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# A cluster of three cases of botulism due to *Clostridium baratii* type F, France, August 2015

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A cluster of three cases of food-borne botulism due to *Clostridium baratii* type F occurred in France in August 2015. All cases required respiratory assistance. Consumption of a Bolognese sauce at the same restaurant was the likely source of contamination. *Clostridium baratii* was isolated both from stool specimens from the three patients and ground meat used to prepare the sauce. This is the second episode reported in France caused by this rare pathogen.

## Description of the cases

In August 2015, two clinically suspected cases of botulism (having both gastrointestinal and neurological symptoms such as dysphagia, diplopia or blurred vision, and progressive paralysis) occurred within the same intensive care unit in France and were reported to the French public health authorities. Patients were from different households. Both had presented with gastrointestinal symptoms two days before hospital admission. Electromyography (EMG) showed a presynaptic block and cerebrospinal fluid (CSF) examination was normal. Both patients received a botulinum antitoxin ABE treatment, four and five days respectively, after symptom onset.

One week later, a third clinically suspected case of botulism with gastrointestinal symptom onset within the same timeframe as the previous two was reported. This patient was initially admitted to a local paediatric unit and later transferred to intensive care unit with dyspnoea, dysphonia and global muscular weakness. He also presented blurred vision and bilateral mydriasis. A few days later, he was transferred to the regional paediatric intensive care unit because of clinical worsening. Guillain-Barré syndrome was initially suspected based on compatible symptoms, elevated CSF protein level, and a first EMG that did not show any presynaptic block (day 5 after symptom onset). Therefore, he was treated with Intravenous immunoglobulin as an atypical Guillain-Barré syndrome. A second EMG showed presynaptic block six days later (day 11 after symptom onset). Because of this late diagnosis and clinical improvement, this patient did not receive any botulinum antitoxin treatment.

None of the three cases had any gastrointestinal disease or invasive gastrointestinal procedure before illness onset.

All patients subsequently developed quadriplegia and respiratory failure requiring intubation and respiratory assistance between 24 and 48 hours after symptom onset. Median length of hospitalisation was 27 days (range: 16–38) and mean duration of intubation was 15.5 days (range: 9–25). All patients recovered.

## Epidemiological, food and environmental investigation

The patients were not related to each other. The only common food item was pasta with Bolognese sauce eaten by the three patients on the same day (lunch or dinner) at the same restaurant in late August. The mean duration from consumption of the suspected meal to symptom onset was 32 hours (range: 18–54 hours) and neurological symptoms developed between 24 and 72 hours after consumption of the suspected meal.

One day after the notification of the first two cases (clinical suspicion), the implicated restaurant was inspected by the district food control authority (DDPP). Inspection revealed hygiene deficiencies in food manufacturing and storage according to good practice guidelines of the national food safety authorities.

**Table**

The National Reference Center for anaerobic bacteria and botulism analysis of 21 food samples, France, August 2015

| Food item | Day of sampling | Presence of toxin | Presence of *Clostridium baratii* |
|---|---|---|---|
| Dried cured ham | D1 | Neg | Neg |
| Anchovies | D1 | Neg | Neg |
| Pesto | D1 | Neg | Neg |
| Ocean sauce (seafood) | D1 | Neg | Neg |
| Frozen ground meat | D3 | Neg | Pos |
| Curry chicken | D3 | Neg | Neg |
| Oil | D3 | Neg | Neg |
| Tomatoes | D3 | Neg | Neg |
| Onions | D3 | Neg | Neg |
| Olive oil | D3 | Neg | Neg |
| Snails | D3 | Neg | Neg |
| Leeks | D3 | Neg | Neg |
| Seafood | D3 | Neg | Neg |
| Defrosted ground meat | D3 | Neg | Pos |
| Capers | D3 | Neg | Neg |
| Tortellini | D3 | Neg | Neg |
| Raviolini | D3 | Neg | Neg |
| Smoked salmon | D3 | Neg | Neg |
| Bolognese sauce[a] | D3 | Neg | Neg |
| Oregano | D3 | Neg | Neg |
| Basque chicken | D3 | Neg | Neg |

Neg: negative; Pos: positive.

[a] Served after the suspected meal.

D1 represents Day 1, one day after the notification of the first two cases.

D3 represents Day 3, three days after the notification of the first two cases.

Samples of food at risk for botulism [1] were collected and sent to the National Reference Center (NRC) for anaerobic bacteria and botulism in Paris (Table). Two days later, although no leftover from the implicated Bolognese sauce was available, a second collection of food samples targeting sauce ingredients was performed (Table).

## Laboratory investigation

The NRC evidenced toxicity in the serum samples of each patient (mouse bioassay) but could not confirm the identification of botulinum toxin type.

In early September, 13 days after symptom onset, *C. baratii* was identified at the NRC in the stool samples of the three patients by PCR and culture. Botulinum toxin F was recovered from stool samples of two patients.

No neurotoxigenic *Clostridium* or toxin was identified in any of the food samples collected during the first inspection (conducted one day after the notification of the first two cases). However, both frozen and defrosted ground meat samples subsequently collected were positive for *C. baratii* but no toxin was detected (Table). In addition, all 34 meat samples collected from stored samples at the meat producer tested negative for *C. baratii* at the NRC.

## Public health measures

One day after the second inspection, the implicated restaurant closed for stock renewal and environmental cleaning. No other consumers of Bolognese sauce were identified and no other botulism case linked to this restaurant was further reported despite the fact that the local emergency department was alerted by the local health authorities.

Trace-back and trace-forward investigation of the contaminated meat batch was conducted by the Ministry of Agriculture (DGAL) which issued a product recall. Although this batch had been distributed to different catering facilities since March 2015, no *C. baratii*-related botulism case had been reported between March and August 2015.

## Discussion

Food-borne botulism due to *C. baratii* type F is rare and only a limited number of cases have been reported worldwide [1-6].

The three cases reported here presented with severe symptoms and rapid progression towards respiratory failure and quadriplegia, although of shorter duration compared with *C. botulinum*-related botulism [4]. These clinical signs are similar to those described for type F botulism cases [1-8]. A high level of protein in the CSF has also been described with botulism [7,9], which could lead to misdiagnosis.

The first two patients received type ABE botulinum antitoxin four and five days respectively, after symptom onset. According to scientific advice on type F botulism, published by the European Centre for Disease Prevention and Control (ECDC), this treatment is expected to be ineffective for type F-related cases [1]. However, it has to be noted that these first two patients had both hospitalisation and intubation duration shorter than the third patient who did not receive any antitoxin. This difference could be due to other factors such as age, medical history, toxin amount ingested or other treatment.

The investigation identified the ground meat used to prepare the sauce as the most probable vehicle of *C. baratii* contamination. However, the ultimate source and mode of contamination of the meat remain unknown. No further case was identified in France during the shelf-life of the contaminated meat despite the wide distribution of the product, and the investigation of the producer's stored samples was negative.

No toxin was found in frozen and defrosted ground meat but the sauce eaten by the patients was not tested. Based on the restaurant inspection results (no

temperature monitoring of stored preparations) and given the known conditions of toxin production, we can hypothesise that the botulinum toxin was produced during the sauce cooking process or storage. Indeed, preparations of a large volume of meat sauce by boiling for more than ten minutes and storage at room temperature for several hours are favourable conditions including anaerobiosis and substrate requirement for *Clostridium* growth and toxin production.

Suspected sources of *C. baratii* botulism reported in the literature included tomato meat sauce [5] and meat pit pies [6].

These types of food and ways of toxin production are not the most common in botulism, which is usually associated with home-canned products or dried pork products [1]. From 1981 to 2002, only nine cases of *C. baratii* botulism in adults were reported in the United States and a food source was implicated in only one [4,5,7]. Hypothesis of adult intestinal colonisation botulism was considered to explain unknown sources, particularly when gastrointestinal factors that can lead to alterations of the intestinal gut flora are associated [4,7]. Since 2002, cases with unidentified sources have been reported in the United States [8,10-12].Finally, some of the unidentified sources for type F botulism could be due to unusual sources such as meat.

This is the second *C. baratii* type F botulism outbreak in France in less than one year [2,3]. Given that strains are centralised at the NRC, we can expect that any new case of type F botulism that may occur in the future, will be identified and notified. The type F botulism due to *C. baratii* represented an emerging issue in France and in Europe. Type F botulism should be suspected in rapid onset and severe flaccid paralysis to promptly confirm the diagnosis with specific tests and rapidly administer appropriate antitoxin as treatment, when available. In Europe, the current botulinum antitoxin (capable of protecting only against type A, B, E toxins) is not appropriate in case of type F botulism, therefore the replacement with the heptavalent antitoxin could be considered.

Assuming that this type of botulism is emerging, it needs to be monitored with caution because investigations of new cases in France and Europe may bring new information about the origin of the contamination by *C. baratii*.


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## Conflict of interest

None declared.

## Authors' contributions

Hélène Tréhard: Collecting data, literature review, wrote the first draft of the paper and reviewed the manuscript critically.

Isabelle Poujol: Collecting data, wrote the first draft of the paper and reviewed the manuscript critically.

Christelle Mazuet: Microbiological analysis, literature review and reviewed the manuscript critically.

Quentin Blanc: Clinical management of patients and reviewed the manuscript critically.

Yves Gillet: Clinical management of patient and reviewed the manuscript critically.

Frédérique Rossignol: Veterinary investigation and reviewed the manuscript critically.

Michel-Robert Popoff: Microbiological analysis, literature review and reviewed the manuscript critically.

Nathalie Jourdan Da Silva: Study conducted, wrote the first draft of the paper and reviewed the manuscript critically.

## References


1. European Centre for Disease Prevention and Control (ECDC). Technical report. Scientific advice on type F botulism. Stockholm: ECDC; Oct 2013. Available from: http://ecdc.europa.eu/en/publications/Publications/botulism-scientific-advice-type-F-botulism.pdf
2. Castor C, Mazuet C, Saint-Leger M, Vygen S, Coutureau J, Durand M, et al. Cluster of two cases of botulism due to Clostridium baratii type F in France, November 2014. Euro Surveill. 2015;20(6):21031. DOI: 10.2807/1560-7917.ES2015.20.6.21031 PMID: 25695475
3. Mazuet C, Sautereau J, Legeay C, Bouchier C, Bouvet P, Jourdan da Silva N, et al. Characterization of the first Clostridium baratii strain responsible for an outbreak of botulism type F in France. Journal of Clinical Microbiology & Case Reports. 2015;1(1):8.
4. Gupta A, Sumner CJ, Castor M, Maslanka S, Sobel J. Adult botulism type F in the United States, 1981-2002. Neurology. 2005;65(11):1694-700. DOI: 10.1212/01.wnl.0000187127.92446.4c PMID: 16344510
5. Harvey SM, Sturgeon J, Dassey DE. Botulism due to Clostridium baratii type F toxin.J Clin Microbiol. 2002;40(6):2260-2. DOI: 10.1128/JCM.40.6.2260-2262.2002 PMID: 12037104
6. Lafuente S, Nolla J, Valdezate S, Tortajada C, Vargas-Leguas H, Parron I, et al. Two simultaneous botulism outbreaks in Barcelona: Clostridium baratii and Clostridium botulinum. Epidemiol Infect. 2013;141(9):1993-5. DOI: 10.1017/S0950268812002592 PMID: 23158693
7. McCroskey LM, Hatheway CL, Woodruff BA, Greenberg JA, Jurgenson P. Type F botulism due to neurotoxigenic Clostridium baratii from an unknown source in an adult.J Clin Microbiol. 1991;29(11):2618-20.PMID: 1774272
8. Sobel J, Dill T, Kirkpatrick CL, Riek L, Luedtke P, Damrow TA. Clinical recovery and circulating botulinum toxin type F in adult patient.Emerg Infect Dis. 2009;15(6):969-71. DOI: 10.3201/eid1506.070571 PMID: 19523306
9. Sonnabend WF, Sonnabend OA, Gründler P, Ketz E. Intestinal toxicoinfection by Clostridium botulinum type F in an adult. Case associated with Guillain-Barré syndrome.Lancet. 1987;1(8529):357-61. DOI: 10.1016/S0140-6736(87)91729-6 PMID: 2880164
10. Centers for Disease Control and Prevention (CDC). National Enteric Disease Surveillance: Botulism Annual Summary, 2005. Available from: http://www.cdc.gov/nationalsurveillance/pdfs/botulism_cste_2005.pdf
11. Centers for Disease Control and Prevention (CDC). National Enteric Disease Surveillance: Botulism Annual Summary, 2007. Available from: http://www.cdc.gov/nationalsurveillance/pdfs/botulism_cste_2007.pdf

12. Centers for Disease Control and Prevention (CDC). National Enteric Disease Surveillance: Botulism Annual Summary, 2013. Atlanta: CDC. 2013. Available from: http://www.cdc.gov/nationalsurveillance/pdfs/botulism_cste_2013.pdf

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# Evaluation of immunochromatographic tests for the rapid detection of the emerging GII.17 norovirus in stool samples, January 2016

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**A novel GII.17 norovirus emerged in Asia in the winter of 2014/15. A worldwide spread is conceivable and norovirus diagnostic assays need to be evaluated to investigate if they adequately detect this emerging genotype. Seven immunochromatographic kits commercially available in Europe were evaluated on ten stool samples where GII.17 virus had been quantified by real-time reverse transcription-polymerase chain reaction. All the kits detected GII.17 with various sensitivities, partly depending on the virus titre.**

We report that seven commercially available norovirus immunochromatographic (IC) tests available in Europe, have the capacity to detect strains of genogroup GII.17 in stool. Sensitivities vary however, and partly depend on the viral load in the samples.

## Laboratory investigation

The following seven IC tests were evaluated on the same panel of stool samples: Actim Noro (Medix Biochemica, Kauniainen, Finland), Immunocatch Norovirus (Eiken Chemical Co., Ltd., Tokyo, Japan), Immunoquick Norovirus (Biosynex S.A., Strasbourg, France), Nadal Norovirus I+II (Nal van minden, Regensburg, Germany), SD Bioline Norovirus (Standard Diagnostics, Inc., Yongin-si, Republic of Korea), Simple Norovirus (Operon, S.A., Cuarte de Huerva, Zaragoza, Spain), and RidaQuick Norovirus (R-Biopharm AG, Darmstadt, Germany).

The assays were performed on 10 frozen stool samples from our collection. These samples had been collected from patients affected by five different gastroenteritis outbreaks. One outbreak was related to oyster consumption, while the four others were caused by person-to-person transmission. Three of the four latter outbreaks had occurred in nursing homes and the fourth in a hospital. All 10 samples had been previously shown to be GII.17 norovirus-positive by sequencing of the RNA-dependent RNA polymerase (RdRp) region and the N-terminal/shell (N/S) region. The samples had also been genotyped on the Norovirus Automated Genotyping Tool [1]. The capsid sequences displayed 95.8% to 99.7% nt identity with the reference strain Hu/GII/JP/2014/GII.P17_GII.17/Kawasaki323 (GenBank accession number: AB983218).

The stool samples were conserved at -40 °C and were thawed on the day of the evaluation. All the commercial IC tests were performed according to the manufacturers' instructions. Due to limited amounts of samples, one assay per test was done, except when the results were negative, in which case they were controlled by a second assay when possible. The virus copy numbers were quantified by real-time reverse transcription-polymerase chain reaction (RT-PCR) on the same day.

Evaluation of the IC tests showed that all were able to detect GII.17 norovirus present in the stool samples. Sensitivities of the tests varied however, ranging from detection in one sample of the 10, to seven samples of the 10 (Table).

Measurements of the virus titres in each sample allowed to demonstrate that a positive result could be obtained with most of the IC tests (five in seven) if the samples presented a minimal load of $4.88 \times 10^8$ virus copies/g of stool. The lowest viral load detected by an IC test was $6.54 \times 10^6$ copies/g of stool and this gave a weak positive signal with the RidaQuick Norovirus test. Of note however, three samples (E13289, E12908, E12909) with relatively high viral loads of $1.35 \times 10^9$, $1.34 \times 10^{10}$ and $3.51 \times 10^{10}$ copies/g of stool gave negative results with four, three and two IC tests, respectively. These samples originated from two separate outbreaks. Another set of samples from two different outbreaks, namely E13289 and E12990 ($6.89 \times 10^8$ copies/g of stool) also yielded different results throughout the IC tests. While E12990 was positive in

**Table**

Results of seven immunochromatographic tests on ten stool samples containing GII.17 norovirus at various concentrations

| Sample ID[a] | Virus titre (copies/g of stool)[b] | Norovirus immunochromatographic tests | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | RidaQuick Norovirus | Simple Norovirus | Immunocatch Norovirus | Actim Noro | Immunoquick Norovirus | SD Bioline Norovirus | NADAL Norovirus I+II |
| E12909 | $3.51 \times 10^{10}$ | Positive | Positive | Positive | Positive | Positive | Negative[c] | Negative |
| E12905 | $1.55 \times 10^{10}$ | Positive | Positive | Positive | Positive | Positive | Positive | Positive |
| E12908 | $1.34 \times 10^{10}$ | Positive | Positive | Positive | Positive | Negative[c] | Negative[c] | Negative |
| E13289 | $1.35 \times 10^{9}$ | Positive | Positive | Positive | Negative[c] | Negative[c] | Negative[c] | Negative |
| E12990 | $6.89 \times 10^{8}$ | Positive | Positive | Positive | Positive | Positive | Positive | Negative |
| E11161 | $4.88 \times 10^{8}$ | Positive | Positive | Positive | Positive | Positive | Negative[c] | Negative |
| E12989 | $9.39 \times 10^{6}$ | Negative[c] | Negative[c] | Negative | Negative[c] | Negative[c] | Negative[c] | Negative |
| E13290 | $6.54 \times 10^{6}$ | Positive | Negative[c] | Negative | Negative[c] | Negative[c] | Negative[c] | Negative |
| E12991 | $4.90 \times 10^{5}$ | Negative[c] | Negative[c] | Negative | Negative[c] | Negative[c] | Negative[c] | Negative |
| E12972 | $1.12 \times 10^{4}$ | Negative[c] | Negative[c] | Negative | Negative[c] | Negative[c] | Negative[c] | Negative |

ID: identity.

[a] E12909, E12905 and E12908 were collected from one single gastroenteritis outbreak; E13289 and E13290 were collected from a second gastroenteritis outbreak; E12990, E12989 and E12991 were collected from a third gastroenteritis outbreak; all three outbreaks occurred in nursing homes. E12972 was collected from an outbreak in a hospital. All four outbreaks were due to a person-to-person transmission, and GII.17 norovirus was the only strain detected. E11161 was collected from an oyster-related gastroenteritis outbreak; GII.17 was the only viral strain detected in this sample.

[b] Quantified by real-time reverse transcription-polymerase chain reaction.

[c] The result was controlled by a second assay.

all but one of the tests, E13289 was negative in four of the IC tests.

On the other hand, some samples, which were common to an outbreak (E12909, E12905 and E12908), and presumably all carrying the same GII.17 strain, also did not react in the same way in three IC tests despite relatively high virus titres (≥$1.34 \times 10^{10}$ copies/g of stool). While they all gave positive results with four IC tests, only two of the three samples gave a positive signal with Immunoquick Norovirus, and only one of the three with SD Bioline Norovirus and Nadal Norovirus I+II.

## Discussion

In the winter of 2014/15 a novel GII.17 norovirus emerged as a major cause of epidemic and endemic acute gastroenteritis in Asia, replacing the previously dominant GII.4 genotype [2-5]. In other parts of the world, GII.17 was up to then only sporadically detected [6-8]. Because noroviruses can spread rapidly around the world, as has been previously observed for GII.4 [9,10], it is possible that GII.17 will emerge in Europe in this season. Thus the public health community and surveillance systems need to be prepared. As emphasised by de Graaf et al. [6], contemporary norovirus diagnostic assays may not have been developed to detect GII.17, since this genotype was rarely found at the time. Therefore they need to be evaluated and adapted if necessary to adequately detect GII.17 norovirus.

For medical laboratories not equipped to carry out molecular investigations, easy-to-perform tests providing rapid results present an advantage. Khamrin et al. [11] tested four IC kits available in Japan for their sensitivity to detect GII.17 strains in faecal specimens. Here we checked the performance of several tests commercially available in Europe.

We found that most of the IC tests could confirm the presence of the GII.17 norovirus, if the samples presented a minimal load of $4.88 \times 10^{8}$ virus copies/g of stool. A study by Takanashi et al. [12] showed that a minimal viral load of $4.6 \times 10^{6}$ copies/g of stool was sufficient to detect GII.4 norovirus by immunochromatography, while a 100-fold higher detection limit was found for GII.17. A similar observation for GII.17 was made by Khamrin et al. [11].

Three samples in our study, all of which had relatively high viral loads (≥$1.35 \times 10^{9}$copies/g of stool) nevertheless gave negative results with some of the tests. Apart from viral load, which is clearly essential for the reactivity of the IC tests against GII.17, other factors may influence the tests' performance. The failure of some IC tests to detect some GII.17 strains could be due to the particular antibodies used in these tests, which may react differently when strains present antigenic variation. This could explain why sample E13289, despite its higher viral load ($1.35 \times 10^{9}$ copies/g of stool), was positive in only three of the IC tests, while sample E12990 with lower viral load ($6.89 \times 10^{8}$ copies/g of stool) was positive in all but one the IC tests. Indeed these two samples originated from different outbreaks and thus may potentially have had antigenic differences. Sequencing the genetic regions coding for the antigens involved in the tests' reactions could further assess this supposition.

Three samples (E12909, E12905 and E12908) originating from the same outbreak however, and thus assumed to contain the same GII.17 strain, reacted differently in three of the IC tests. Therefore, viral loads and antigenic differences cannot explain these results. One alternative reason could be the consistence of the stool samples paired with the sample collection devices. Indeed, the sample collection devices of the seven IC tests are not the same, and it is possible that some of them are not appropriate to collect certain stool samples. This hypothesis nevertheless remains to be confirmed.

Some limitations of this study should be mentioned. In particular, the possibility of false negatives for samples not controlled by a second assay cannot be excluded. Furthermore, even if this was not the objective of this study, it should be noted that a precise estimation of the detection limit of each IC test by serial dilutions of the samples was not performed.

In conclusion, the seven IC tests evaluated were able to detect GII.17 with various sensitivities due to virus titre, and possibly antigenic differences and kit design. Therefore some IC tests may need to be optimised for the detection of GII.17.


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## Conflict of interest

None declared.

## Authors' contributions

LT: conducted the laboratory investigation; MB: conducted the laboratory investigation; PP: revised the manuscript; KAB: conceptualised the study and drafted the manuscript.

## References


1. Kroneman A, Vennema H, Deforche K, v d Avoort H, Peñaranda S, Oberste MS, et al. An automated genotyping tool for enteroviruses and noroviruses. J Clin Virol. 2011;51(2):121-5. DOI: 10.1016/j.jcv.2011.03.006 PMID: 21514213
2. Fu J, Ai J, Jin M, Jiang C, Zhang J, Shi C, et al. Emergence of a new GII.17 norovirus variant in patients with acute gastroenteritis in Jiangsu, China, September 2014 to March 2015. Euro Surveill. 2015;20(24):21157. DOI: 10.2807/1560-7917.ES2015.20.24.21157 PMID: 26111236
3. Lu J, Sun L, Fang L, Yang F, Mo Y, Lao J, et al. Gastroenteritis Outbreaks Caused by Norovirus GII.17, Guangdong Province, China, 2014-2015. Emerg Infect Dis. 2015;21(7):1240-2. DOI: 10.3201/eid2107.150226 PMID: 26080037
4. Matsushima Y, Ishikawa M, Shimizu T, Komane A, Kasuo S, Shinohara M, et al. Genetic analyses of GII.17 norovirus strains in diarrheal disease outbreaks from December 2014 to March 2015 in Japan reveal a novel polymerase sequence and amino acid substitutions in the capsid region. Euro Surveill. 2015;20(26):21173. DOI: 10.2807/1560-7917.ES2015.20.26.21173 PMID: 26159307
5. Han J, Ji L, Shen Y, Wu X, Xu D, Chen L. Emergence and predominance of norovirus GII.17 in Huzhou, China, 2014-2015. Virol J. 2015;12(1):139. DOI: 10.1186/s12985-015-0370-9 PMID: 26362650
6. de Graaf M, van Beek J, Vennema H, Podkolzin AT, Hewitt J, Bucardo F, et al. Emergence of a novel GII.17 norovirus – End of the GII.4 era? Euro Surveill. 2015;20(26):21178. DOI: 10.2807/1560-7917.ES2015.20.26.21178 PMID: 26159308
7. Medici MC, Tummolo F, Calderaro A, Chironna M, Giammanco GM, De Grazia S, et al. Identification of the novel Kawasaki 2014 GII.17 human norovirus strain in Italy, 2015. Euro Surveill. 2015;20(35):30010. DOI: 10.2807/1560-7917.ES.2015.20.35.30010 PMID: 26530698
8. Parra GI, Green KY. Genome of Emerging Norovirus GII.17, United States, 2014.Emerg Infect Dis. 2015;21(8):1477-9. DOI: 10.3201/eid2108.150652 PMID: 26196235
9. Eden JS, Hewitt J, Lim KL, Boni MF, Merif J, Greening G, et al. The emergence and evolution of the novel epidemic norovirus GII.4 variant Sydney 2012. Virology. 2014;450-451:106-13. DOI: 10.1016/j.virol.2013.12.005 PMID: 24503072
10. Siebenga JJ, Vennema H, Zheng DP, Vinjé J, Lee BE, Pang XL, et al. Norovirus illness is a global problem: emergence and spread of norovirus GII.4 variants, 2001-2007. J Infect Dis. 2009;200(5):802-12. DOI: 10.1086/605127 PMID: 19627248
11. Khamrin P, Thongprachum A, Takanashi S, Okitsu S, Maneekarn N, Hayakawa S, et al. Evaluation of immunochromatography tests for detection of novel GII.17 norovirus in stool samples. Euro Surveill. 2015;20(28):21185. DOI: 10.2807/1560-7917.ES2015.20.28.21185 PMID: 26212141
12. Takanashi S, Okame M, Shiota T, Takagi M, Yagyu F, Tung PG, et al. Development of a rapid immunochromatographic test for noroviruses genogroups I and II. J Virol Methods. 2008;148(1-2):1-8. DOI: 10.1016/j.jviromet.2007.10.010 PMID: 18054091


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# Differential age susceptibility to influenza B/Victoria lineage viruses in the 2015 Australian influenza season

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**Influenza B viruses make up an important part of the burden from seasonal influenza globally. The 2015 season in Australia saw an unusual predominance of influenza B with a distinctive switch during the season from B/Yamagata/16/88 lineage viruses to B/Victoria/2/87 lineage viruses. We also noted significant differences in the age groups infected by the different B lineages, with B/Victoria infecting a younger population than B/Yamagata, that could not be explained by potential prior exposure.**

The 2015 season was notable for the predominance of influenza B in Australia. According to the Australian Influenza Surveillance Report [1] for the period 1 January to 9 October, 61% of cases were typed as influenza B and 38% influenza A (29% A (not subtyped), 7% A(H3N2) and 2% A(H1N1)pdm09). That season was also interesting due to the waxing and waning of the two B lineages over the season. Here, we summarise the lineage distribution using viruses submitted to the WHO Collaborating Centre for Reference and Research on Influenza in Melbourne for 2015 and compared these data with data from 2008, the last year when influenza B viruses predominated in Australia.

## The 2015 influenza season in Australia

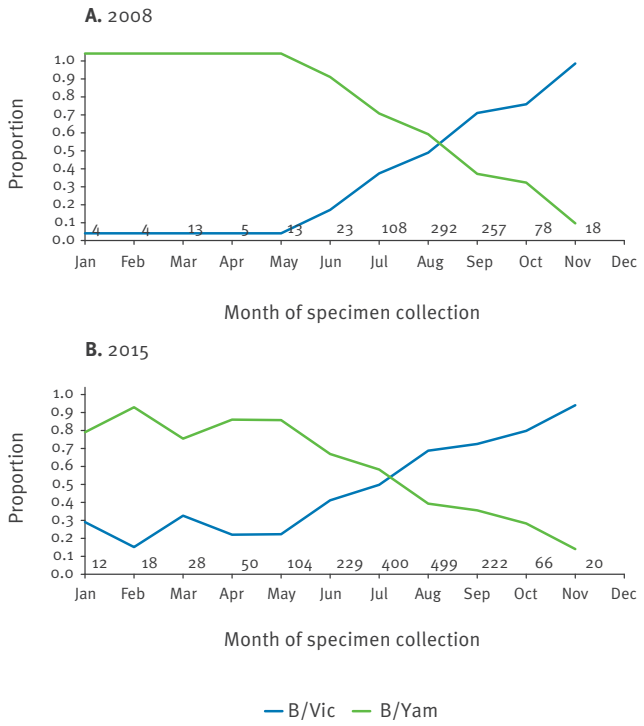
Lineage data was available for 816 influenza B viruses from 2008 and 1,648 from 2015 that were received by the Centre from all over Australia. The formal representativeness of these samples is unknown. Generally there is a bias towards sampling from children and this is seen in most years in most general and sentinel surveillance systems and was also seen in the 2008 [2] and 2015 [1] Australian influenza seasons. It is unlikely that any bias would exist in selecting patients with a particular B lineage, and given the size and the geographical diversity of the samples tested, it is likely these data will provide an accurate estimate of the overall situation with influenza B in Australia during these two years. During the 2015 pre-season period (January–April) and the early part of the influenza season (May–June), B/Yamagata/16/88 lineage (B/Yam) viruses predominated. However, from July to November, B/Victoria/2/87 lineage (B/Vic) viruses increased rapidly and were dominant from August (Figure 1). Notably, this same switch was seen during the 2008 season with similar timing although almost no B/Vic lineage viruses were detected in Australia before June (Figure 1). The distributions of lineages during 2015 were similar when individual Australian states were examined, with the exception of the Northern Territory, which has a small population largely situated in the tropics, that had an almost total B/Vic year (36/39 B viruses). Australia's most populous state, New South Wales, experienced an increase in the proportion of B/Vic viruses from low levels early in 2015 to 28% during the period from 15 June to 12 July 2015 [3] which according to our study continued to increase over the rest of the influenza season, and B/Vic viruses predominated from July onwards. Children and young adults carry a higher burden of influenza B disease than older adults and the elderly. According to the Australian Paediatric Surveillance Unit 2015 saw 88 children 15 years and younger (median: 3.3 years) hospitalised with severe complications of influenza between 1 July 2015 and 30 September 2015. Roughly two thirds (n = 59) were influenza B cases (lineage unknown) [1]. Overall, the average duration of hospitalisation was four days, 20 required an ICU admission, and there were three influenza-associated deaths, all associated with influenza B infections [1].

## Antigenic and genetic drift

The move in dominance by the B/Vic viruses in 2015 was not accompanied by any major antigenic changes from the B/Vic viruses that circulated in smaller numbers in 2014. All Australian B/Vic viruses analysed by the Centre were antigenically B/Brisbane/60/2008-like as they were in 2014 (data not shown). Equally, the 2015 B/Yam viruses that were analysed remained

**Figure 1**

Relative frequency of influenza B subtypes received by month of specimen collection, Australia, 2008 (n = 816) and 2015 (n = 1,648)




B/Vic: B/Victoria/2/87 lineage; B/Yam: B/Yamagata/16/88 lineage.

Numbers at the bottom of the figure are the total number of B viruses tested for each month. Top panel: influenza B subtypes in 2008 (n=415 B/Vic, n=401 B/Yam); bottom panel: influenza B subtypes in 2015 (n=852 B/Vic, n=796 B/Yam).

antigenically B/Phuket3073/2013-like, similar to B/Yam viruses that circulated in Australia from mid-2014 (data not shown). However, subtle phylogenetic differences in the haemagglutinin (HA) genes of the two B lineages were apparent (Figure 2). The HA gene phylogeny revealed a greater diversity for B/Yam viruses isolated in Australia during 2015 (n=56) than during 2014 (n=42) (Figure 2, top panel). The mean time to most recent common ancestor (mTMRCA) extended beyond 2012 and the isolates belonged to three antigenic types (B/Wisconsin/1/2010, B/Massachusetts/2/2012 and B/Phuket/3073/2013), although the majority from 2015 (52 of the 56 viruses from 2015) belonged to the B/Phuket/3073/2013 clade (also known as group 3) with a mTMRCA in mid-2014. Reconstruction of non-synonymous changes along this phylogeny revealed an amino acid substitution (M267V) in the subclade of B/Phuket/3073/2013-like viruses that were dominant during the 2015 season, suggesting that this mutation may have contributed to increased viral fitness. All Australian B/Vic viruses from 2015 (n=54) were phylogenetically B/Brisbane/60/2008-like (also referred to as clade 1A) with an mTMRCA in 2013. This clade was made up of three distinct subclades (Figure 2, bottom panel) the largest of which had the non-synonymous amino acid substitutions V161I and I132V that may have also enhanced the fitness of these viruses.

## Age distribution of influenza B infections

The age distribution of patients with confirmed B/Vic infections in 2015 was positively skewed, with a greater number of infections among the younger age groups (mean: 26.4 years, median: 19.9 years). For B/Yam infections, the age distribution was more even (mean: 42.4 years, median: 43.8 years; $p<0.001$ for Wilcoxon rank sum test). This age differential was less evident in 2008 where, despite the high proportion of viruses obtained from children younger than five years, the interquartile range indicated that B/Yam viruses affected a broader age range than the B/Vic viruses (Figure 3). This age difference between lineages has previously been reported from a household study in Hong Kong [4]. There, children younger than 15 years had a 13-fold increased risk of secondary influenza infection with a B/Vic virus than with a B/Yam virus, during a period when both lineages were co-circulating. Similar findings were reported in population studies in southern China during the 2009 and 2010 seasons [5], over three seasons in Slovenia (2010–13 [6]) and in our earlier studies from eastern Australia and New Zealand, where major differences in lineage distribution were observed in subjects older than five years [5,7].

## Discussion

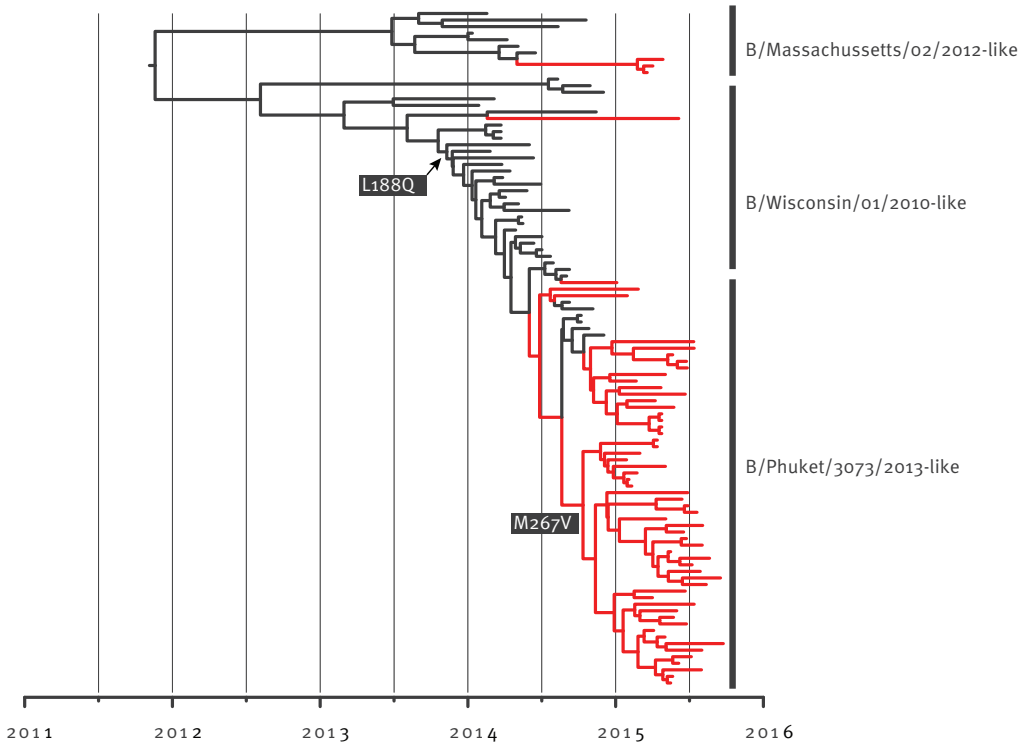
A predominance of influenza B viruses in an influenza season occurs infrequently, usually in the order of once every 10 years. Prior to 2015, it last occurred in Australia in the 2008 season, where 54% of typed viruses were influenza B, 43% were influenza A and 3% untyped [2]. Similarly in Europe for the seasons from 2001/02 to 2010/11, influenza B was the majority influenza type (59.1%) in only one season (2005/06). In the United States (US) over the same period, 2002/03 was the season with the highest proportion of influenza B (42.6%) among of all typed viruses [8]. The two antigenic and genetically distinct lineages of influenza B viruses (B/Yam and B/Vic) have co-circulated in various proportions since 2002 in most countries. Trivalent influenza vaccines (containing only one B virus lineage) used over this time have tried to match these changing lineage circulation patterns. Because of a number of poor matches during the 2000s, quadrivalent vaccines (containing viruses from both B lineages) were developed and have recently been introduced in order to improve vaccine effectiveness. The 2015 influenza vaccines licensed in Australia were all traditional inactivated virus vaccines (live attenuated influenza vaccines and recombinant vaccines were not available) with mostly trivalent vaccine containing only the B/Yam component being used along with low levels of quadrivalent vaccine.

The reasons for the apparent differential age susceptibility between the two B lineages described in this

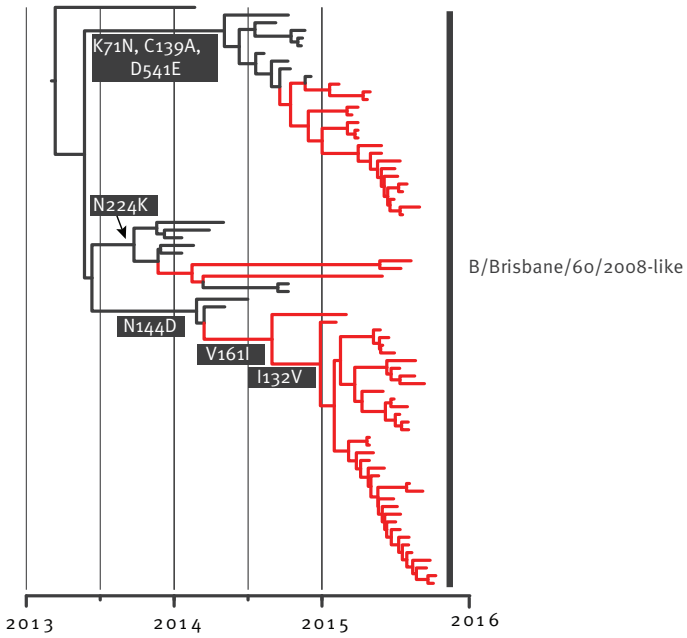
## Figure 2

Maximum clade credibility trees showing the evolution of haemagglutinin genes of sequenced Australian influenza B viruses from 2014 and 2015 (n = 168)

**A.** Yamagata




**B.** Victoria




Phylogenies were inferred using a relaxed molecular clock model in a Bayesian Markov Chain Monte Carlo framework with the programme BEASTv1.8 [15].

Panel A: B/Yamagata/16/88 lineage viruses isolated in Australia in 2014 (n=42) and 2015 (n=56); Panel B: B/Victoria/2/87 lineage viruses isolated in Australia in 2014 (n=16) and 2015 (n=54). Red bars: Australian influenza B viruses from 2015; black bars: Australian influenza B viruses from 2014. Non-synonymous amino acid changes that occurred during the evolution of the lineages are shown adjacent to the nodes. All available haemagglutinin (HA) sequences were obtained from The Global Initiative on Sharing All Influenza Data (GISAID; http://platform.gisaid.org/epi3/frontend). See the Table at the end of the article for details of the source and details of the virus and the sequencing laboratory.

## Table a

Australian influenza B viruses and haemagglutinin gene sequences used to construct Figure 2, obtained from The Global Initiative on Sharing All Influenza Data (GISAID)[a]

| Segment ID | Collection date | Isolate name | Influenza B lineage | Originating laboratory | Authors[b] |
|---|---|---|---|---|---|
| EPI551283 | 2014-Aug-11 | B/Newcastle/21/2014 | Victoria | John Hunter Hospital | A |
| EPI561891 | 2014-Dec-05 | B/Darwin/43/2014 | Victoria | Royal Darwin Hospital | A |
| EPI541294 | 2014-Feb-18 | B/Tasmania/1/2014 | Victoria | Royal Hobart Hospital | A |
| EPI541365 | 2014-Feb-21 | B/Perth/503/2014 | Victoria | Pathwest | A |
| EPI529392 | 2014-Jan-20 | B/Brisbane/3/2014 | Victoria | QHSS | A |
| EPI540771 | 2014-Jan-20 | B/Perth/501/2014 | Victoria | Pathwest | A |
| EPI551321 | 2014-Jul-02 | B/South Australia/20/2014 | Victoria | IMVS | A |
| EPI540747 | 2014-Mar-29 | B/Brisbane/12/2014 | Victoria | QHSS | A |
| EPI551327 | 2014-May-03 | B/Sydney/19/2014 | Victoria | Clinical Virology Unit, CDIM | A |
| EPI541291 | 2014-May-06 | B/Brisbane/13/2014 | Victoria | QHSS | A |
| EPI562018 | 2014-Nov-14 | B/Brisbane/71/2014 | Victoria | QHSS | A |
| EPI561888 | 2014-Nov-20 | B/Brisbane/74/2014 | Victoria | QHSS | A |
| EPI561873 | 2014-Oct-09 | B/Brisbane/62/2014 | Victoria | QHSS | A |
| EPI561876 | 2014-Oct-10 | B/Brisbane/63/2014 | Victoria | QHSS | A |
| EPI561924 | 2014-Oct-15 | B/Victoria/7/2014 | Victoria | VIDRL | A |
| EPI551336 | 2014-Sep-09 | B/Victoria/204/2014 | Victoria | Royal Chidrens Hospital | A |
| EPI636426 | 2015-Apr-23 | B/Darwin/9/2015 | Victoria | Royal Darwin Hospital | B |
| EPI636340 | 2015-Apr-28 | B/Brisbane/46/2015 | Victoria | QHSS | B |
| EPI636409 | 2015-Apr-30 | B/Darwin/11/2015 | Victoria | Royal Darwin Hospital | B |
| EPI675691 | 2015-Aug-02 | B/Victoria/849/2015 | Victoria | Austin Health | B |
| EPI675652 | 2015-Aug-03 | B/South Australia/1036/2015 | Victoria | IMVS | B |
| EPI648854 | 2015-Aug-03 | B/Victoria/847/2015 | Victoria | Austin Health | B |
| EPI675636 | 2015-Aug-04 | B/Newcastle/1012/2015 | Victoria | IMVS | B |
| EPI675677 | 2015-Aug-05 | B/Victoria/1009/2015 | Victoria | IMVS | B |
| EPI675663 | 2015-Aug-09 | B/Sydney/137/2015 | Victoria | Westmead Hospital | B |
| EPI648856 | 2015-Aug-11 | B/Victoria/861/2015 | Victoria | Austin Health | B |
| EPI675694 | 2015-Aug-18 | B/Victoria/898/2015 | Victoria | Austin Health | B |
| EPI675672 | 2015-Aug-20 | B/Tasmania/30/2015 | Victoria | Royal Hobart Hospital | B |
| EPI675646 | 2015-Aug-30 | B/Perth/201/2015 | Victoria | Pathwest | B |
| EPI630025 | 2015-Feb-05 | B/Brisbane/4/2015 | Victoria | QHSS | A |
| EPI630050 | 2015-Feb-12 | B/South Australia/3/2015 | Victoria | IMVS | A |
| EPI636504 | 2015-Jul-02 | B/South Australia/1015/2015 | Victoria | IMVS | B |
| EPI648850 | 2015-Jul-06 | B/Townsville/7/2015 | Victoria | QHSS | B |
| EPI636421 | 2015-Jul-07 | B/Darwin/17/2015 | Victoria | Royal Darwin Hospital | B |
| EPI648882 | 2015-Jul-12 | B/Victoria/524/2015 | Victoria | Monash Medical Centre | B |
| EPI636621 | 2015-Jul-12 | B/Victoria/525/2015 | Victoria | Monash Medical Centre | B |
| EPI675604 | 2015-Jul-13 | B/Brisbane/186/2015 | Victoria | QHSS | B |
| EPI648868 | 2015-Jul-13 | B/Canberra/27/2015 | Victoria | Canberra Hospital | B |
| EPI648846 | 2015-Jul-14 | B/Brisbane/185/2015 | Victoria | QHSS | B |
| EPI675639 | 2015-Jul-15 | B/Newcastle/28/2015 | Victoria | John Hunter Hospital | B |
| EPI648870 | 2015-Jul-19 | B/Canberra/29/2015 | Victoria | Canberra Hospital | B |
| EPI636388 | 2015-Jul-19 | B/Canberra/30/2015 | Victoria | Canberra Hospital | B |
| EPI648848 | 2015-Jul-30 | B/Darwin/22/2015 | Victoria | Royal Darwin Hospital | B |
| EPI675688 | 2015-Jul-31 | B/Victoria/843/2015 | Victoria | Austin Health | B |

IMVS: Institute of Medical and Veterinary Science; Pathwest: Pathwest QE II Medical Centre; QHSS: Queensland Health Scientific Services; VIDRL: Victoria Infectious Diseases Laboratory.

[a] All samples were sequenced and submitted by WHO Collaborating Centre for Reference and Research on Influenza, Melbourne, Australia, with the exception of B/Brisbane/47/2015 that was submitted by US Centers for Disease Control and Prevention.

[b] Authors: A: Deng Y-M, Iannello P, Spirason N, Jelley L, Lau H, Komadina N; B: Deng Y-M, Iannello P, Spirason N, Lau H, Komadina N; C: Tilmanis D, Hurt A, Komadina N.

## Table B

Australian influenza B viruses and haemagglutinin gene sequences used to construct Figure 2, obtained from The Global Initiative on Sharing All Influenza Data (GISAID)[a]

| Segment ID | Collection date | Isolate name | Influenza B lineage | Originating laboratory | Authors[b] |
|---|---|---|---|---|---|
| EPI636549 | 2015-Jun-01 | B/Sydney/11/2015 | Victoria | Clinical Virology Unit, CDIM | B |
| EPI636525 | 2015-Jun-04 | B/South Australia/49/2015 | Victoria | IMVS | B |
| EPI636567 | 2015-Jun-15 | B/Tasmania/2/2015 | Victoria | Royal Hobart Hospital | B |
| EPI636329 | 2015-Jun-27 | B/Brisbane/136/2015 | Victoria | QHSS | B |
| EPI636415 | 2015-Jun-28 | B/Darwin/14/2015 | Victoria | Royal Darwin Hospital | B |
| EPI636635 | 2015-Jun-28 | B/Victoria/557/2015 | Victoria | Monash Medical Centre | B |
| EPI636577 | 2015-Jun-29 | B/Tasmania/5/2015 | Victoria | Royal Hobart Hospital | B |
| EPI636465 | 2015-Jun-30 | B/Newcastle/1005/2015 | Victoria | IMVS | B |
| EPI636560 | 2015-Mar-02 | B/Sydney/503/2015 | Victoria | Prince of Wales Hospital | B |
| EPI636334 | 2015-Mar-16 | B/Brisbane/15/2015 | Victoria | QHSS | B |
| EPI636584 | 2015-Mar-31 | B/Townsville/3/2015 | Victoria | QHSS | B |
| EPI636605 | 2015-Mar-31 | B/Victoria/502/2015 | Victoria | Monash Medical Centre | B |
| EPI636354 | 2015-May-08 | B/Brisbane/55/2015 | Victoria | QHSS | B |
| EPI636361 | 2015-May-21 | B/Brisbane/69/2015 | Victoria | QHSS | B |
| EPI636363 | 2015-May-24 | B/Brisbane/70/2015 | Victoria | QHSS | B |
| EPI636369 | 2015-May-25 | B/Brisbane/73/2015 | Victoria | QHSS | B |
| EPI636485 | 2015-May-28 | B/Perth/24/2015 | Victoria | Pathwest | B |
| EPI636488 | 2015-May-28 | B/Perth/25/2015 | Victoria | Pathwest | B |
| EPI636658 | 2015-May-30 | B/South Australia/48/2015 | Victoria | IMVS | B |
| EPI636472 | 2015-May-31 | B/Newcastle/7/2015 | Victoria | John Hunter Hospital | B |
| EPI675619 | 2015-Oct-03 | B/Darwin/65/2015 | Victoria | Royal Darwin Hospital | B |
| EPI675622 | 2015-Oct-09 | B/Darwin/70/2015 | Victoria | Royal Darwin Hospital | B |
| EPI675660 | 2015-Sep-07 | B/Sydney/1071/2015 | Victoria | IMVS | B |
| EPI675655 | 2015-Sep-11 | B/South Australia/118/2015 | Victoria | IMVS | B |
| EPI675602 | 2015-Sep-16 | B/Brisbane/1036/2015 | Victoria | IMVS | B |
| EPI675686 | 2015-Sep-25 | B/Victoria/700/2015 | Victoria | Monash Medical Centre | B |
| EPI540782 | 2014-Apr-04 | B/Newcastle/3/2014 | Yamagata | John Hunter Hospital | A |
| EPI540744 | 2014-Apr-08 | B/Darwin/35/2014 | Yamagata | Royal Darwin Hospital | A |
| EPI540765 | 2014-Apr-15 | B/Sydney/8/2014 | Yamagata | Prince of Wales Hospital | A |
| EPI540762 | 2014-Apr-29 | B/Sydney/7/2014 | Yamagata | Prince of Wales Hospital | A |
| EPI551286 | 2014-Aug-11 | B/Newcastle/22/2014 | Yamagata | John Hunter Hospital | A |
| EPI551289 | 2014-Aug-12 | B/Newcastle/25/2014 | Yamagata | John Hunter Hospital | A |
| EPI561915 | 2014-Dec-02 | B/Sydney/39/2014 | Yamagata | Westmead Hospital | A |
| EPI562030 | 2014-Dec-03 | B/Perth/579/2014 | Yamagata | Pathwest | A |
| EPI529622 | 2014-Feb-17 | B/Townsville/3/2014 | Yamagata | QHSS | A |
| EPI540779 | 2014-Feb-25 | B/Perth/505/2014 | Yamagata | Pathwest | A |
| EPI529377 | 2014-Jan-12 | B/Darwin/4/2014 | Yamagata | Royal Darwin Hospital | A |
| EPI529619 | 2014-Jan-28 | B/Brisbane/4/2014 | Yamagata | QHSS | A |
| EPI551274 | 2014-Jul-03 | B/Newcastle/12/2014 | Yamagata | John Hunter Hospital | A |
| EPI540912 | 2014-Jul-03 | B/South Australia/21/2014 | Yamagata | IMVS | A |
| EPI551280 | 2014-Jul-24 | B/Newcastle/19/2014 | Yamagata | John Hunter Hospital | A |
| EPI551324 | 2014-Jul-31 | B/Sydney/1002/2014 | Yamagata | IMVS | A |
| EPI541331 | 2014-Jun-02 | B/Brisbane/22/2014 | Yamagata | QHSS | A |
| EPI541279 | 2014-Jun-12 | B/South Australia/16/2014 | Yamagata | IMVS | A |
| EPI541338 | 2014-Jun-13 | B/Newcastle/8/2014 | Yamagata | John Hunter Hospital | A |

IMVS: Institute of Medical and Veterinary Science; Pathwest: Pathwest QE II Medical Centre; QHSS: Queensland Health Scientific Services; VIDRL: Victoria Infectious Diseases Laboratory.

[a] All samples were sequenced and submitted by WHO Collaborating Centre for Reference and Research on Influenza, Melbourne, Australia, with the exception of B/Brisbane/47/2015 that was submitted by US Centers for Disease Control and Prevention.

[b] Authors: A: Deng Y-M, Iannello P, Spirason N, Jelley L, Lau H, Komadina N; B: Deng Y-M, Iannello P, Spirason N, Lau H, Komadina N; C: Tilmanis D, Hurt A, Komadina N.

## Table c

Australian influenza B viruses and haemagglutinin gene sequences used to construct Figure 2, obtained from The Global Initiative on Sharing All Influenza Data (GISAID)[a]

| Segment ID | Collection date | Isolate name | Influenza B lineage | Originating laboratory | Authors[b] |
|---|---|---|---|---|---|
| EPI551277 | 2014-Jun-17 | B/Newcastle/17/2014 | Yamagata | John Hunter Hospital | A |
| EPI540909 | 2014-Jun-30 | B/South Australia/1002/2014 | Yamagata | IMVS | A |
| EPI541241 | 2014-Mar-07 | B/Brisbane/8/2014 | Yamagata | QHSS | A |
| EPI551249 | 2014-Mar-24 | B/Brisbane/9/2014 | Yamagata | QHSS | A |
| EPI540759 | 2014-Mar-26 | B/Sydney/5/2014 | Yamagata | Prince of Wales Hospital | A |
| EPI540785 | 2014-Mar-28 | B/Newcastle/5/2014 | Yamagata | John Hunter Hospital | A |
| EPI540906 | 2014-May-05 | B/Sydney/13/2014 | Yamagata | Westmead Hospital | A |
| EPI540768 | 2014-May-06 | B/Sydney/9/2014 | Yamagata | Prince of Wales Hospital | A |
| EPI540753 | 2014-May-14 | B/South Australia/5/2014 | Yamagata | IMVS | A |
| EPI540756 | 2014-May-17 | B/South Australia/7/2014 | Yamagata | IMVS | A |
| EPI541288 | 2014-May-27 | B/South Australia/1000/2014 | Yamagata | IMVS | A |
| EPI561918 | 2014-Nov-06 | B/Victoria/512/2014 | Yamagata | Monash Medical Centre | A |
| EPI561885 | 2014-Nov-11 | B/Brisbane/70/2014 | Yamagata | QHSS | A |
| EPI630034 | 2014-Nov-14 | B/Canberra/20/2014 | Yamagata | Canberra Hospital | A |
| EPI561870 | 2014-Oct-08 | B/Brisbane/61/2014 | Yamagata | QHSS | A |
| EPI561912 | 2014-Oct-08 | B/Perth/569/2014 | Yamagata | Pathwest | A |
| EPI561879 | 2014-Oct-20 | B/Brisbane/65/2014 | Yamagata | QHSS | A |
| EPI561882 | 2014-Oct-27 | B/Brisbane/66/2014 | Yamagata | QHSS | A |
| EPI561921 | 2014-Oct-30 | B/Victoria/6/2014 | Yamagata | VIDRL | A |
| EPI551820 | 2014-Sep-02 | B/Victoria/804/2014 | Yamagata | Austin Health | A |
| EPI551330 | 2014-Sep-08 | B/Townsville/1000/2014 | Yamagata | IMVS | A |
| EPI551264 | 2014-Sep-09 | B/Darwin/38/2014 | Yamagata | Royal Darwin Hospital | A |
| EPI551333 | 2014-Sep-09 | B/Victoria/202/2014 | Yamagata | Royal Chidrens Hospital | A |
| EPI636392 | 2015-Apr-02 | B/Canberra/4/2015 | Yamagata | Canberra Hospital | B |
| EPI636341 | 2015-Apr-03 | B/Brisbane/33/2015 | Yamagata | QHSS | B |
| EPI630067 | 2015-Apr-05 | B/Victoria/500/2015 | Yamagata | Monash Medical Centre | A |
| EPI636553 | 2015-Apr-09 | B/Sydney/5/2015 | Yamagata | Clinical Virology Unit, CDIM | B |
| EPI642630 | 2015-Apr-14 | B/Brisbane/47/2015 | Yamagata | WHO CC | NA |
| EPI636506 | 2015-Apr-23 | B/South Australia/12/2015 | Yamagata | IMVS | B |
| EPI636606 | 2015-Apr-25 | B/Victoria/503/2015 | Yamagata | Monash Medical Centre | B |
| EPI648860 | 2015-Apr-25 | B/Victoria/530/2015 | Yamagata | Monash Medical Centre | B |
| EPI636345 | 2015-Apr-28 | B/Brisbane/50/2015 | Yamagata | QHSS | B |
| EPI648852 | 2015-Aug-01 | B/Victoria/845/2015 | Yamagata | Austin Health | B |
| EPI675669 | 2015-Aug-02 | B/Sydney/70/2015 | Yamagata | Westmead Hospital | B |
| EPI675644 | 2015-Aug-03 | B/Perth/166/2015 | Yamagata | Pathwest | B |
| EPI675657 | 2015-Aug-04 | B/Sydney/1031/2015 | Yamagata | IMVS | B |
| EPI675666 | 2015-Aug-13 | B/Sydney/153/2015 | Yamagata | Westmead Hospital | B |
| EPI675675 | 2015-Aug-21 | B/Tasmania/32/2015 | Yamagata | Royal Hobart Hospital | B |
| EPI630031 | 2015-Feb-03 | B/Canberra/1/2015 | Yamagata | Canberra Hospital | A |
| EPI630047 | 2015-Feb-10 | B/South Australia/2/2015 | Yamagata | IMVS | C |
| EPI630053 | 2015-Feb-21 | B/South Australia/4/2015 | Yamagata | IMVS | A |
| EPI630055 | 2015-Feb-23 | B/South Australia/5/2015 | Yamagata | IMVS | C |
| EPI630064 | 2015-Feb-26 | B/Townsville/1/2015 | Yamagata | QHSS | A |
| EPI630016 | 2015-Jan-04 | B/Brisbane/1/2015 | Yamagata | QHSS | A |

IMVS: Institute of Medical and Veterinary Science; Pathwest: Pathwest QE II Medical Centre; QHSS: Queensland Health Scientific Services; VIDRL: Victoria Infectious Diseases Laboratory.

[a] All samples were sequenced and submitted by WHO Collaborating Centre for Reference and Research on Influenza, Melbourne, Australia, with the exception of B/Brisbane/47/2015 that was submitted by US Centers for Disease Control and Prevention.

[b] Authors: A: Deng Y-M, Iannello P, Spirason N, Jelley L, Lau H, Komadina N; B: Deng Y-M, Iannello P, Spirason N, Lau H, Komadina N; C: Tilmanis D, Hurt A, Komadina N.

## Table d

Australian influenza B viruses and haemagglutinin gene sequences used to construct Figure 2, obtained from The Global Initiative on Sharing All Influenza Data (GISAID)[a]

| Segment ID | Collection date | Isolate name | Influenza B lineage | Originating laboratory | Authors[b] |
|---|---|---|---|---|---|
| EPI630061 | 2015-Jan-28 | B/Sydney/2/2015 | Yamagata | Clinical Virology Unit, CDIM | A |
| EPI630058 | 2015-Jan-30 | B/Sydney/1000/2015 | Yamagata | IMVS | A |
| EPI636636 | 2015-Jul-07 | B/Victoria/543/2015 | Yamagata | Monash Medical Centre | B |
| EPI636618 | 2015-Jul-09 | B/Victoria/519/2015 | Yamagata | Monash Medical Centre | B |
| EPI636601 | 2015-Jul-13 | B/Victoria/32/2015 | Yamagata | VIDRL | B |
| EPI636387 | 2015-Jul-14 | B/Canberra/28/2015 | Yamagata | Canberra Hospital | B |
| EPI636627 | 2015-Jul-14 | B/Victoria/532/2015 | Yamagata | Monash Medical Centre | B |
| EPI636641 | 2015-Jul-21 | B/Victoria/952/2015 | Yamagata | Royal Chidrens Hospital | B |
| EPI675641 | 2015-Jul-29 | B/Perth/136/2015 | Yamagata | Pathwest | B |
| EPI636592 | 2015-Jun-01 | B/Victoria/301/2015 | Yamagata | Melbourne Pathology | B |
| EPI636531 | 2015-Jun-06 | B/South Australia/50/2015 | Yamagata | IMVS | B |
| EPI636566 | 2015-Jun-06 | B/Tasmania/1/2015 | Yamagata | Royal Hobart Hospital | B |
| EPI636313 | 2015-Jun-14 | B/Brisbane/100/2015 | Yamagata | QHSS | B |
| EPI636322 | 2015-Jun-18 | B/Brisbane/118/2015 | Yamagata | QHSS | B |
| EPI636460 | 2015-Jun-22 | B/Newcastle/1003/2015 | Yamagata | IMVS | B |
| EPI636468 | 2015-Jun-22 | B/Newcastle/20/2015 | Yamagata | John Hunter Hospital | B |
| EPI636326 | 2015-Jun-25 | B/Brisbane/132/2015 | Yamagata | QHSS | B |
| EPI636379 | 2015-Jun-25 | B/Canberra/13/2015 | Yamagata | Canberra Hospital | B |
| EPI636535 | 2015-Jun-25 | B/South Australia/71/2015 | Yamagata | IMVS | B |
| EPI636574 | 2015-Jun-27 | B/Tasmania/4/2015 | Yamagata | Royal Hobart Hospital | B |
| EPI636383 | 2015-Jun-28 | B/Canberra/15/2015 | Yamagata | Canberra Hospital | B |
| EPI636541 | 2015-Jun-29 | B/Sydney/1013/2015 | Yamagata | IMVS | B |
| EPI630019 | 2015-Mar-02 | B/Brisbane/11/2015 | Yamagata | QHSS | A |
| EPI630022 | 2015-Mar-20 | B/Brisbane/19/2015 | Yamagata | QHSS | A |
| EPI636455 | 2015-May-03 | B/Newcastle/1/2015 | Yamagata | John Hunter Hospital | B |
| EPI636349 | 2015-May-04 | B/Brisbane/54/2015 | Yamagata | QHSS | B |
| EPI636515 | 2015-May-04 | B/South Australia/22/2015 | Yamagata | IMVS | B |
| EPI636514 | 2015-May-05 | B/South Australia/18/2015 | Yamagata | IMVS | B |
| EPI636500 | 2015-May-17 | B/South Australia/1000/2015 | Yamagata | IMVS | B |
| EPI636611 | 2015-May-17 | B/Victoria/507/2015 | Yamagata | Monash Medical Centre | B |
| EPI636482 | 2015-May-22 | B/Perth/21/2015 | Yamagata | Pathwest | B |
| EPI636521 | 2015-May-22 | B/South Australia/28/2015 | Yamagata | IMVS | B |
| EPI636593 | 2015-May-25 | B/Townsville/6/2015 | Yamagata | QHSS | B |
| EPI675616 | 2015-Sep-17 | B/Darwin/61/2015 | Yamagata | Royal Darwin Hospital | B |
| EPI675683 | 2015-Sep-24 | B/Victoria/698/2015 | Yamagata | Monash Medical Centre | B |

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[a] All samples were sequenced and submitted by WHO Collaborating Centre for Reference and Research on Influenza, Melbourne, Australia, with the exception of B/Brisbane/47/2015 that was submitted by US Centers for Disease Control and Prevention.

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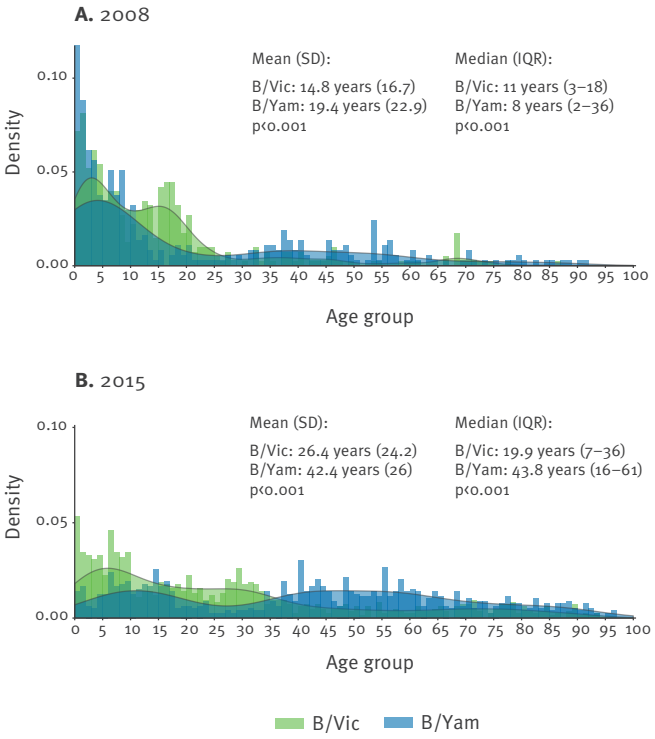
**Figure 3**

Age distribution of confirmed influenza B cases by lineage, Australia, 2008 (n = 780) and 2015 (n = 1,638)




IQR: interquartile range; SD: standard deviation.

Data shown are samples for which age data was available. Bars show the histogram in one-year age increments by lineage. The shaded areas indicate the smoothed density estimate of the age distribution. Values provided on the graphs for each lineage are mean (SD) or median (IQR). P values are for the t-test comparing mean age between the two lineages and the Wilcoxon rank-sum test for comparing medians.

study are unknown. It is, however, well known that the different influenza types/subtypes do affect different age profiles; both seasonal and 2009 pandemic A(H1N1) as well as influenza B viruses infect a younger population than A(H3N2) viruses [9], although in recent years, the median age of influenza A(H1N1)pdm09 cases has been increasing [6,10], again for unknown reasons. Studies to date have not shown differences in clinical presentation for the different B lineages [4,5], but long-term data on hospitalisations and deaths are lacking. In a study by Paddock et al. on deaths attributed to confirmed influenza B in the US from 2000 to 2010, the majority of subjects were 18 years and younger (34/45 cases), and a slightly higher proportion of infections were B/Vic compared with B/Yam (25 vs 17 deaths, respectively, in those cases that could be characterised) [11]. More studies are required to determine if there is indeed any difference in outcomes following severe infections with either of the B lineages in different age groups.

It is probable that the prior exposure history of the different age groups has influenced our observations. However, this is difficult to deduce from the present data. For example, five-year-old Australian children in 2015 were likely to have been exposed to a mixture of B/Yam viruses, which circulated in 2013 and 2014, and B/Vic viruses, which circulated from 2009 to 2012, as was the case in for five-year-olds in 2008. Possible exposure therefore fails to explain the elevated proportion of five year-old children infected with B/Yam viruses in 2008 or with B/Vic in 2015. In addition, it is unlikely given the low levels of childhood vaccination in Australia that this this would have significantly altered the circulation patterns of the influenza B lineages. Vaccination uptake is generally below 10% among Australian children [12]. Childhood influenza vaccination is only recommended for children of aboriginal descent five years and younger and for children six months and older with comorbidities [13].

We have suggested previously that there may be some fundamental differences in the receptor specificity of the different influenza B lineages and that the distribution or density of receptors for influenza B viruses in the respiratory tract of humans may differ with age [7]. Others have shown differential responses of children to B/Yam and B/Vic antigens contained in influenza vaccines that might also contribute to differential susceptibility to these two lineages 14]. Further work is needed to fully understand the basis of these observations and to determine if the differences are due to receptor variation or density during ageing or prior exposure history or a mixture of both. If indeed young children are at an elevated risk of infection with B/Vic viruses, then it may be prudent to prioritise distribution of quadrivalent vaccines (containing viruses from both B lineages) to this age group. This is relevant to the current northern hemisphere influenza season where the trivalent vaccine contains a B/Yam lineage virus, but B/Vic lineage viruses are in our view likely to increase substantially during the current season. Use of the quadrivalent vaccine for this subgroup (or preferably for the whole population) would be potentially advantageous in improving influenza vaccine effectiveness.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## Conflict of interest

None declared.

## Authors' contributions

IB and SS wrote the manuscript, SS performed the epidemiological analysis, DV performed phylogenetic analyses, all authors revised the manuscript.

## References


1. Australian Government Department of Health. Australian Influenza Surveillance Report. No.10, 2015, reporting period: 26 September to 9 October 2015. Canberra: Australian Government Department of Health; 2015. Available from: http://www.health.gov.au/internet/main/publishing.nsf/Content/cda-surveil-ozflu-flucurr.htm/$File/Australian-Influenza-Surveillance-Report.pdf
2. Kaczmarek M, Owen R, Barr IG. Annual report of the National Influenza Surveillance Scheme, 2008.Commun Dis Intell Q Rep. 2010;34(1):8-22.PMID: 20521494
3. Jennings Z, Carter I, McPhie K, Kok J, Dwyer DE. Increased prevalence of influenza B/Victoria lineage viruses during early stages of the 2015 influenza season in New South Wales, Australia: implications for vaccination and planning. Euro Surveill. 2015;20(31):21201. DOI: 10.2807/1560-7917.ES2015.20.31.21201 PMID: 26290426
4. Xu C, Chan KH, Tsang TK, Fang VJ, Fung RO, Ip DK, et al. Comparative Epidemiology of Influenza B Yamagata- and Victoria-Lineage Viruses in Households. Am J Epidemiol. 2015;182(8):705-13. DOI: 10.1093/aje/kwv110 PMID: 26400854
5. Tan Y, Guan W, Lam TT, Pan S, Wu S, Zhan Y, et al. Differing epidemiological dynamics of influenza B virus lineages in Guangzhou, southern China, 2009-2010. J Virol. 2013;87(22):12447-56. DOI: 10.1128/JVI.01039-13 PMID: 24027322
6. Sočan M, Prosenc K, Učakar V, Berginc N. A comparison of the demographic and clinical characteristics of laboratory-confirmed influenza B Yamagata and Victoria lineage infection.J Clin Virol. 2014;61(1):156-60. DOI: 10.1016/j.jcv.2014.06.018 PMID: 25034374
7. Vijaykrishna D, Holmes EC, Joseph U, Fourment M, Su YC, Halpin R, et al. The contrasting phylodynamics of human influenza B viruses. eLife. 2015;4:e05055. DOI: 10.7554/eLife.05055 PMID: 25594904
8. Ambrose CS, Levin MJ. The rationale for quadrivalent influenza vaccines.Hum Vaccin Immunother. 2012;8(1):81-8. DOI: 10.4161/hv.8.1.17623 PMID: 22252006
9. Kelly HA, Grant KA, Williams S, Fielding J, Smith D. Epidemiological characteristics of pandemic influenza H1N1 2009 and seasonal influenza infection.Med J Aust. 2009;191(3):146-9.PMID: 19645642
10. Skowronski DM, Chambers C, Sabaiduc S, Janjua NZ, Li G, Petric M, et al. Pre- and postpandemic estimates of 2009 pandemic influenza A(H1N1) seroprotection to inform surveillance-based incidence, by age, during the 2013-2014 epidemic in Canada. J Infect Dis. 2015;211(1):109-14. DOI: 10.1093/infdis/jiu366 PMID: 24973459
11. Paddock CD, Liu L, Denison AM, Bartlett JH, Holman RC, Deleon-Carnes M, et al. Myocardial injury and bacterial pneumonia contribute to the pathogenesis of fatal influenza B virus infection. J Infect Dis. 2012;205(6):895-905. DOI: 10.1093/infdis/jir861 PMID: 22291193
12. Sullivan SG, Chilver MB, Higgins G, Cheng AC, Stocks NP. Influenza vaccine effectiveness in Australia: results from the Australian Sentinel Practices Research Network.Med J Aust. 2014;201(2):109-11. DOI: 10.5694/mja14.00106 PMID: 25045991
13. Australian Government Department of Health and Ageing. Part 4 Vaccine-preventable diseases. The Australian Immunisation Handbook. 10th ed. Canberra: National Health and Medical Research Council; 2015. p. 251-6. Available from: http://www.immunise.health.gov.au/internet/immunise/publishing.nsf/Content/immunise-influenza (Accessed 20 Jan. 2016)
14. Skowronski DM, Hottes TS, De Serres G, Ward BJ, Janjua NZ, Sabaiduc S, et al. Influenza B/Victoria antigen induces strong recall of B/Yamagata but lower B/Victoria response in children primed with two doses of B/Yamagata. Pediatr Infect Dis J. 2011;30(10):833-9. DOI: 10.1097/INF.0b013e31822db4dc PMID: 21857263
15. Drummond AJ, Suchard MA, Xie D, Rambaut A. Bayesian phylogenetics with BEAUti and the BEAST 1.7.Mol Biol Evol. 2012;29(8):1969-73. DOI: 10.1093/molbev/mss075 PMID: 22367748


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# Insights into epidemiology of human parvovirus B19 and detection of an unusual genotype 2 variant, Bulgaria, 2004 to 2013

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**The present study aimed to determine the role of human parvovirus B19 (B19V) as an aetiological agent in measles and rubella negative fever/rash patients from Bulgaria between 2004 and 2013. A total of 1,266 sera from all over the country were tested for B19V IgM antibodies and all positives were further investigated by polymerase chain reaction (PCR). Overall, 280 sera (22%) were B19V IgM positive and 227 of these (81%) were also PCR positive. The highest number of IgM positives was found among five to nine year-old children (27%). Eight infected women gave birth to healthy children; one fetus was aborted with hydrops fetalis. Of the 55 genetic sequences obtained, 54 belonged to genotype 1a and one grouped as a genotype 2 outlier. Phylogenetic analysis of all available genotype 2 sequences covering the 994 nucleotide non-structural protein 1(NS1)/capsid viral protein 1 (VP1) unique region junction, showed that only one other sequence grouped with the outlier strain, forming a clearly distinct and well-supported cluster of genotype 2 (between-group genetic distance: 3.32%). In accordance with B19V nomenclature, this cluster may represent a new subgenotype 2b. The study showed that B19V infections may be falsely identified as rubella or measles in ca 22% of cases, emphasising the need for laboratory confirmation.**

## Introduction

Human parvovirus B19 (B19V) belongs to the family *Parvoviridae*, subfamily *Parvovirinae,* genus *Erythrovirus* [1]. The viral genome consists of 5,596 nucleotides (nt) encoding among others the non-structural protein 1 (NS1) and the capsid viral protein 1 (VP1). Phylogenetic analysis of a 994 nt fragment of the NS1/VP1 unique region junction (NS1/VP1u) identified three genotypes (1, 2, and 3) of B19V with no clear differences in clinical outcome [2].

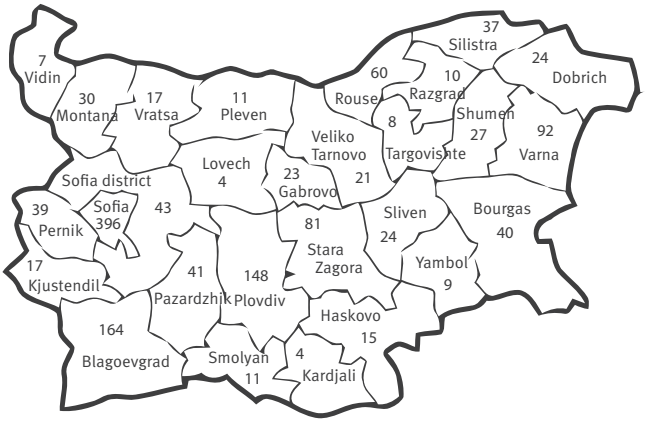
B19V infections are associated with different clinical presentations and although typically a mild, self-limiting disease, the infection can cause severe adverse outcomes in certain patients. The main clinical manifestations are erythema infectiosum also known as fifth disease; transient aplastic crisis may occur in individuals with haemoglobinopathies, chronic anaemia in the immunocompromised, acute polyarthralgia syndrome in adults, and sometimes spontaneous abortion and stillbirth after infection during pregnancy [3,4].

There is no vaccine or antiviral drug to prevent B19V infection. In Bulgaria, as well as in most other countries, this infection is not a notifiable disease. However, the clinical presentation of B19V infection is sometimes mistakenly diagnosed as rubella or measles [3-5], which are both notifiable diseases in Bulgaria. An accurate diagnosis of fever/rash illness is necessary for case management and public health control activities [6], in particular in the context of the World Health Organization's goal to eliminate measles and rubella in the European Region [7,8]. However, in outbreak situations many cases reported as measles or rubella are still not laboratory confirmed in Bulgaria. The laboratory diagnosis of B19V infection in fever (›38.5°C)/rash cases combining serological and molecular methods was introduced in Bulgaria in 2011.

The aim of this mostly retrospective study was to determine the role of B19V as an aetiological agent in measles and rubella negative individuals with fever/rash in

**Figure 1**

Geographical origin, by districts, of samples from rash/fever patients who were measles and rubella IgM-negative, Bulgaria, 2004–2013 (n=1,266)




In the Figure, within each district, the number of samples obtained is shown together with the district name.

Bulgaria, also during rubella and measles outbreaks in the period between 2004 and 2013.

## Methods

### Clinical samples

Serum samples from 1,266 measles and rubella IgM-negative patients between one and 47 years of age received at the National Reference Laboratory for Measles, Mumps and Rubella between 2004 and 2013 were included in the study. The sera were from 654 females (52%) and 612 males (48%). Only the first or single samples from measles/rubella suspected patients with fever/rash illness were tested for B19V. The samples were collected as part of measles/rubella surveillance in Bulgaria with the cooperation of regional hospitals and regional public health agencies from all 28 districts in the country (Figure 1). The majority of the samples (n=1,025, 81%) were from eight districts: Sofia city (n=396), Blagoevgrad (n=164), Plovdiv (n=148), Stara Zagora (n=81), Varna (n=92), Rousse (n=60), Sofia district (n=43) and Pazardzhik (n=41) (Figure 1). Besides age, sex and origin of the patients, disease diagnosis and complications, date of rash onset and of specimen collection and for women of childbearing age also pregnancy status and where appropriate gestational age were recorded. In case of B19V IgM and/or polymerase chain reaction (PCR) positivity in pregnancy, the pregnancy outcome was monitored. For some patients data on vaccination status against measles and/or rubella and travel history were available.

### Laboratory analysis

All serum samples were tested for parvovirus B19 IgM antibodies with a commercial indirect enzyme-linked immunosorbent assay (Mikrogen, recomWell Parvovirus B19 IgM). This assay was found to have a high sensitivity of 76.2% and a specificity of 92.5% (Biotrin: 52.4% and 99.5%, respectively) [9]. The assay was performed and interpreted as recommended by the manufacturer and the results were qualitatively categorised as positive, negative or equivocal.

Viral DNA extraction was attempted from all IgM positive serum samples using the NucleoSpin Blood test kits (Macherey-Nagel GmbH & Co. KG). Screening for B19V DNA was performed with primers e1905f and e1987r targeting a region of the NS1 gene (NS1-PCR) [2] and KAPA Taq PCR kits (Kapa Biosystems, Inc.). Positive results were confirmed with a second PCR assay using primers e2717f and e2901r located in the VP1 unique region (VP1u)-PCR [2]. The NS1-PCR has been shown to amplify all three genotypes of B19V and to have a sensitivity threshold of 200 copies of B19V DNA per ml of sample. Sensitivity, specificity and strain distinction of the VP1u-PCR were reported as being similar to those of the NS1-PCR [2]. Viral DNA for sequencing was prepared by nested-PCR amplification of a 1,100-bp region spanning the NS1/VP1u junction [2]. Amplification of additional parts of the B19V genome was attempted for one outlier strain using previously published primers [10]. Each PCR included negative and positive controls. The PCR products were analysed by electrophoresis in 1.5 or 2% agarose gels stained with ethidium bromide. Products for sequencing were purified with the QIAquick PCR purification kits (Qiagen). NS1/VP1u PCR positive samples were sequenced with the BigDye Terminator v3.1 cycle sequencing kit from Life Technologies. Sequences were edited using SeqScape v2.7 and then aligned with references in BioEdit v7.1. The new sequences are available under European Nucleotide Archive accession numbers LN680930–84.

### Phylogenetic analysis

Phylogenetic analyses as well as distance calculations were performed using Molecular Evolutionary Genetics Analysis (MEGA) version 4.0.2 software [11]. For genotyping, phylogenetic trees were constructed based on 994 nt sequences of the NS1/VP1u of human parvovirus B19 [2]. Further analysis of a genotype 2 strain obtained in this study was based on a sequence of 4,070 nt, corresponding to nt positions 845 to 4,914 of the human parvovirus B19 reference sequence (GenBank accession number: NC_000883.2). The Kimura 2-parameter model and the neighbour-joining algorithm of MEGA4 were applied and only bootstrap values ≥70% (1,000 replicates) were considered significant and are shown in the trees.

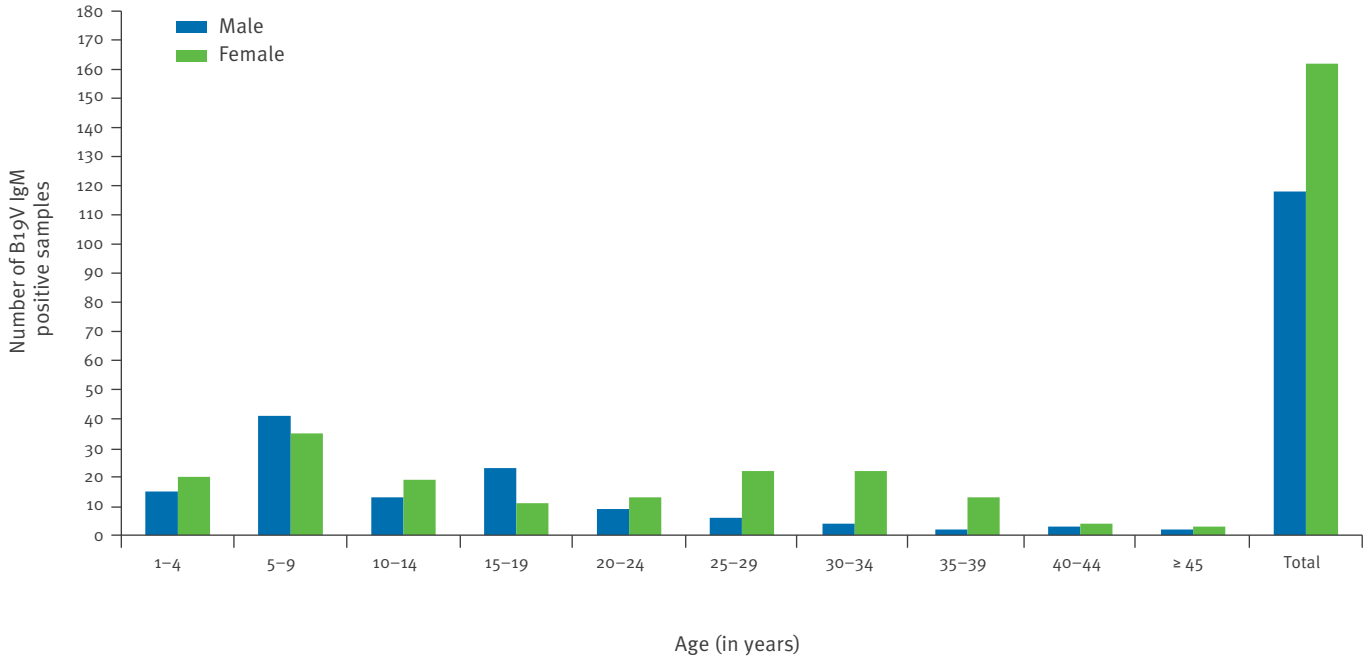
## Results

### Epidemiological findings

From 3,872 samples obtained between 2004 and 2013 from individuals with rash/fever, 1,320 were double negative for measles and rubella. Of these, 1,266 with enough leftover material were further tested for B19V IgM antibodies. Most samples investigated were either

**Figure 2**

Number of human parvovirus B19 IgM positive samples according to age group and sex, Bulgaria, 2004–2013 (n = 280)




B19V: human parvovirus B19.

collected during a rubella outbreak between 2005 and 2006 (568/1,266; 45%) or during a measles outbreak from 2009 to 2010 (378/1,266; 30%) (Table 1) [12,13].

A total of 280 of the 1,266 patients (22%) were B19V IgM positive (Table 1). The highest percentage of positives was detected in 2013, when more than half of the measles/rubella IgM double negative samples tested were positive for B19V (23/45; 51%), (Table 1). The lowest percentage (7/126; 6%) was observed in 2009 (Table 1). Over the whole study period, the highest number of positive samples were from five to nine year-old children (76/280; 27%) and the lowest from adults over44 years-old (5/280, 2%, Figure 2). More females (n=162; 58%) than males (42%) were B19V IgM positive – corresponding to 25% (162/654) of all females and 19% (118/612) of all males –, especially among the 25 to 39 year-olds (57 females vs 12 males IgM positive in this age group). Only among five to nine year-old children and 15 to 19 year-olds, were males more affected (Figure 2).

Our cohort comprised 32 pregnant women (aged 20–24 years: 2; 25–29 years: 14; 30–34 years: 11; 35–39 years: 5), nine of whom were B19V IgM and DNA positive (25–29 years: 5; 30–34 years: 3; 35–39 years: 1). While eight of these women gave birth to healthy children, one patient developed complications (hydrops fetalis) and the fetus was aborted.

Overall, 227 of 280 B19V IgM positive samples (81%) were positive in the NS1 PCR, while 81 (29%) and 109 (39%) were positive in the VP1u and NS1/VP1u PCR, respectively (Table 1).

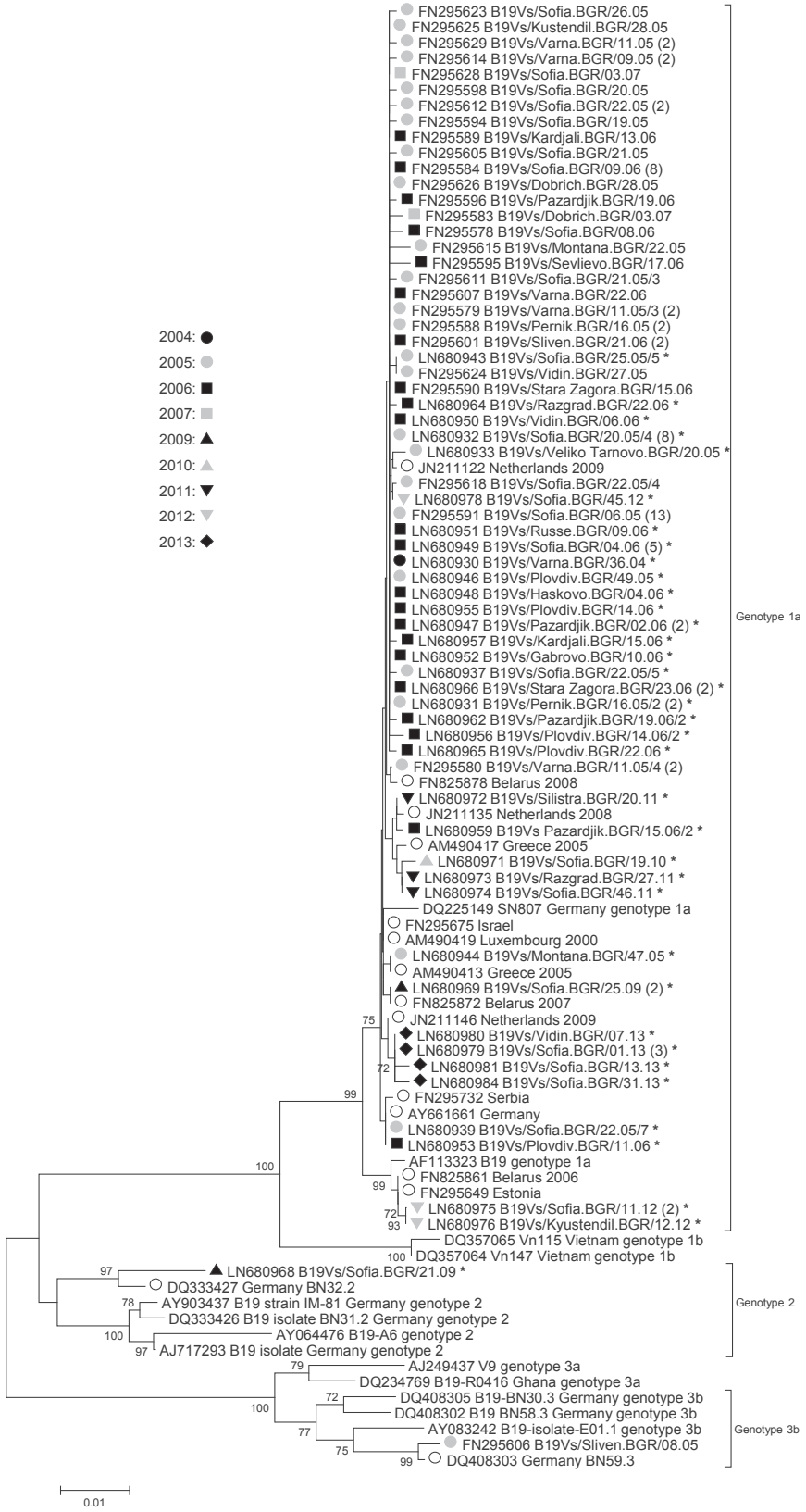
## Genotyping and phylogenetic analyses

NS1/VP1u PCR fragments for sequencing and genotyping were available for all years except 2008 (Table 1). While 54 of the 109 NS1/VP1u PCR positive samples had been sequenced and genotyped before (53 times genotype 1a and 1 time genotype 3b) [14], the remaining 55 products were sequenced and genotyped in the present study. All new sequences belonged to genotype 1a, except for one from a female patient in her 30s with complications of reactive arthritis. This sequence grouped as an outlier to the genotype 2 reference sequences (Figure 3). Within genotype 1a, 20 different sequence variants were found (Figure 3). Taking all sequences from Bulgaria from between 2004 until 2013 into account, there was no clear grouping according to the place of origin. All sequences from 2009 (except for the genotype 2 outlier) grouped together as did the sequences from the period between 2010 and 2011, from early 2012 and from 2013. Except for the group formed by 2010 and 2011 sequences, which contained also a strain detected in 2006, all these groups were separated from sequences of other years (Figure 3).

The overall maximum genetic distance was 13.12%. Without the genotype 3b and genotype 2 outlier sequences, this distance was 1.38% (Table 2). The highest maximum and mean genetic distances within genotype 1a sequences were found in 2012 (respectively 1.12% and 0.55%).

## Figure 3

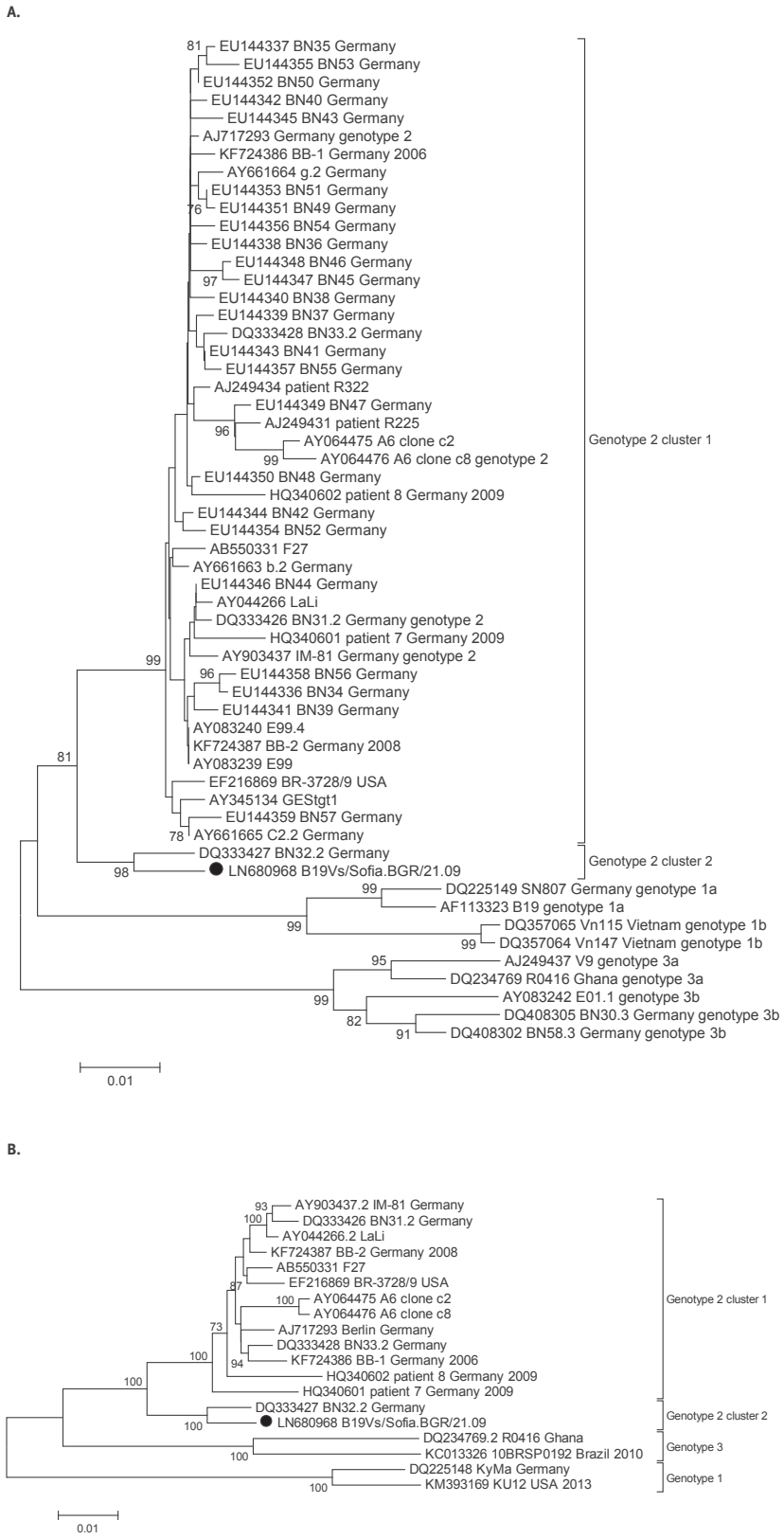
Phylogenetic analysis of the Bulgarian human parvovirus B19 sequences identified in this study, 2004–2013




GenBank numbers and provenance of all sequences used in the tree are indicated. All sequences obtained from Bulgaria between 2004 and 2013 are marked with icons according to the year of sample collection. Such sequences are additionally named with location, epidemiological week and year, and the sequences obtained during the present study are highlighted with an asterisk (*). Only one representative strain is shown for identical sequences from the same year and location, separate for previously published and new sequences in this study, and the total number of identical sequences is indicated in brackets. Similar or identical strains from other countries downloaded from GenBank are identified by open circles and reference sequences are identified by accession number, name and genotype.

## Figure 4

Phylogenetic analyses of a human parvovirus B19 genotype 2 strain recovered in Bulgaria




The sequence from Bulgaria is marked with a black dot and is designated by GenBank number, location, epidemiological week and year. GenBank numbers and provenance of all sequences in the trees are indicated.

A. Analysis of a 994 nt sequence of the Bulgarian strain covering the non-structural protein 1 (NS1)/capsid viral protein 1 (VP1) unique region junction.

B. Analysis of a 4,070 nt sequence of the Bulgarian strain, corresponding to nt positions 845 to 4,914 of the human parvovirus B19 reference sequence (GenBank accession number: NC_000883.2).

**Table 1**

Samples tested for human parvovirus B19 IgM antibodies and DNA and results according to year, Bulgaria, 2004–2013 (n=1,266)

| Year of sample collection | Proportion of samples from patients with fever and rash, which were negative for both measles and rubella n/N | Number of B19V IgM positives per sera tested n/N (%) | Number of NS1 PCR positives[a] n | Number of VP1u PCR positives[a] n | Number of NS1/VP1u PCR positives[a] n |
|---|---|---|---|---|---|
| 2004 | 66/93 | 9/66 (14) | 8 | 1 | 1 |
| 2005 | 405/929 | 89/394 (23) | 74 | 22 | 52 |
| 2006 | 182/322 | 82/174 (47) | 70 | 27 | 37 |
| 2007 | 41/201 | 9/41 (22) | 3 | 1 | 2 |
| 2008 | 42/92 | 9/42 (21) | 3 | 2 | 0 |
| 2009 | 132/610 | 7/126 (6) | 3 | 3 | 3 |
| 2010 | 261/1,390 | 32/252 (13) | 27 | 6 | 1 |
| 2011 | 77/106 | 10/77 (13) | 9 | 3 | 3 |
| 2012 | 54/56 | 10/49 (20) | 9 | 9 | 4 |
| 2013 | 60/73 | 23/45 (51) | 21 | 7 | 6 |
| **Total proportions n/N (%)** | **1,320 [b] /3,872 (34)** | **280/1,266 [b] (22)** | **227/280 (81)** | **81/280 (29)** | **109/280 (39)** |

B19V: human parvovirus B19; NS1: non-structural protein 1; NS1/VP1u: NS1/capsid viral protein 1 unique region junction; PCR: polymerase chain reaction; VP1u: capsid viral protein 1 unique region.

[a] Only B19V IgM positive samples were tested by PCR.

[b] From the 1,320 samples of patients who tested negative for both measles and rubella, 1,266 had enough material left over for investigating B19V.

Phylogenetic analysis of all genotype 2 sequences covering the 994 nt NS1/VP1u region downloaded from GenBank showed that only one other sequence (BN32.2, DQ333427) grouped with the outlier strain, forming a clearly distinct cluster of genotype 2 (Figure 4a). The within group mean was 1.06% for cluster 1 and 1.65% for cluster 2. The between group distance was 3.32%. Phylogenetic analysis using a 4,070 nt region of B19V confirmed the grouping of the outlier strain with sequence BN32.2 as a clearly separate cluster of genotype 2 (Figure 4b).

## Discussion

The present study was based on samples collected during a ten-year period (2004–2013) throughout the country, providing a comprehensive overview of B19V infections during years with widely variable incidences of measles and rubella. Overall, 22% of the fever/rash cases investigated were B19V IgM positive, a relatively high percentage compared with similar studies from e.g. Ireland (4.5%) [3] and England (17%) [15], but lower than the 35.5% reported between 2005 and 2008 from Belarus [16]. Discrepancies between studies may result from the selection of study cohort, design and year(s), measles/rubella control in the country and its geographical location determining the prevalence of different rash/fever causing agents [16-19]. While the lowest yearly proportions of B19V IgM positives in the present study were detected during the 2009 to 2010 measles outbreak (6% and 13%), a high proportion of more than 47% was found towards the end of the 2005 to 2006 rubella outbreak. A recently published study based on a somewhat different patient cohort and only 194 samples collected early during the 2005 to 2006 rubella outbreak, found an even higher rate of IgM positivity of 48.97% [5]. The more similar clinical presentation of rubella and B19V infections, but also the increased B19V incidence reported from elsewhere in Europe in 2005 and 2006 [3,16] may explain the apparent positivity rate difference during the measles and rubella outbreaks. The high rate of B19V IgM positives in 2013 (51%) may be due to a strengthened control of measles and rubella in Bulgaria in light of the 2015 WHO elimination goals in the European Region. Even in the absence of measles and rubella, countries are supposed to analyse samples from suspected cases and to discard at least two suspected cases per 100,000 population per year as non-measles or non-rubella to demonstrate the sensitivity of their surveillance system [8]. With a reduced number of measles and rubella cases, the positive predictive value of positive IgM results decreases. Additional laboratory tests such as viral nucleic acid detection by PCR, IgG titre changes in acute vs convalescent sera or IgG avidity testing may become necessary to confirm IgM test results. Our finding that B19V infections may be falsely identified as rubella or to a lesser extent as measles in ca 22% of the cases is similar to what was reported recently from Belarus [19]. It highlights the benefit of laboratory confirmation of suspected measles/rubella cases, also in outbreak situations.

The highest rate of B19V IgM positivity was found among five to nine year-old children (27%), which is similar to observations from previous studies in

**Table 2**

Genetic distances per year and overall between sequences of human parvovirus B19 detected in Bulgaria, 2004–2013

| Year | Number of sequences | Maximum genetic distance[a] % | Mean genetic distance[a] % |
|---|---|---|---|
| 2004 | 1 | NA | NA |
| 2005 | 52 (51) | 13.12 (0.56) | 0.56 (0.10) |
| 2006 | 37 | 0.61 | 0.09 |
| 2007 | 2 | 0.20 | 0.20 |
| 2009 | 3 (2) | 7.54 (0) | 5.03 (0) |
| 2010 | 1 | – | – |
| 2011 | 3 | 0.10 | 0.07 |
| 2012 | 4 | 1.12 | 0.55 |
| 2013 | 6 | 0.41 | 0.14 |
| **Overall** | **109 (107)** | **13.12 (1.38)** | **0.56 (0.21)** |

NA: not applicable.

[a] In case sequences other than genotype 1a were found in a given year, the values for the genotype 1a sequences only are shown in brackets.

Europe, namely from Belarus [16] and Belgium, England and Wales, Finland, Italy and Poland [18]. More females than males were B19V IgM positive, especially in the 25 to 39 year-olds, possibly because of a high awareness of congenital complications of fever/rash diseases during pregnancy [20,21] and/or because of their closer contacts with children. The PCR targeting the NS1 conservative region detected B19V DNA in more than 81% of IgM positive patients, suggesting that this PCR is of value for the confirmation of B19V infection. Our PCR positivity rate among IgM positives was relatively high compared with the 62% (18/29) reported among rubella and toxoplasmosis suspected but negative patients from Brazil [22] and the 71% (10/14) reported during a B19V outbreak in the Netherlands [23]. Potential explanations for the discrepancies include false positive IgM tests as well as false negative PCR results. The other two PCRs used in our study were less sensitive. At least for the genotyping PCR this may be due to the considerably longer PCR fragment, which is much less likely to be amplified in case of DNA degradation resulting from prolonged sample storage and repeated and long-term defrosting.

The vast majority of the sequences characterised in this study belonged to genotype 1 (53/54, 98%), similar to what has been reported previously from Bulgaria [14]. Although the rate of B19V IgM positivity varied considerably between the years, cases were detected throughout the ten-year period, suggesting an ongoing endemic virus circulation. However, some level of virus clustering by years (in particular during recent years) may indicate repeated virus importations. Genotype 1 is also the most prevalent in most other countries throughout Europe and beyond [14,24-26] and there are recent reports of genotype 3, e.g. from France [2], Greece [14], and the United Kingdom [27]. In contrast, genotype 2 seems to be rare. Infectious B19V genotype 2 variants were identified after organ transplantation [28] and in blood/plasma donations [29,30] in Germany as well as in immunocompromised patients from Poland [31]. A comprehensive study published in 2006 found genotype 2 only in tissues of patients born before 1973 and suggested that this genotype has in principle disappeared from circulation [32]. We found genotype 2 only in one patient born in Bulgaria in the early 1970s and no information about recent travel, transfusion or transplantation history or immunosuppression of the patient was available and no identical strains were found on GenBank. Thus the origin of the strain detected in 2009 remains unclear.

Phylogenetic analysis of all genotype 2 sequences available on GenBank showed that the Bulgarian strain was an outlier forming together with a strain from a German patient (co-infected with genotype 1) [33] a clearly separate cluster of genotype 2 sequences. Both sequences are 98% identical in the 994 nt region and show a between-group genetic distance of 3.32% to the other genotype 2 sequences. Since in addition high bootstrap values are obtained for both clusters and the node linking them, cluster 1 may in accordance with parvovirus B19 nomenclature [34,35] be referred to as subgenotype 2a and cluster 2 as subgenotype 2b.

The introduction of B19V screening of measles and rubella negative specimens from fever/rash patients provided valuable information about the impact of this disease in Bulgaria and will support fever/rash surveillance in the wake of the measles and rubella elimination.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## Conflict of interest

None declared.

## Authors' contributions

SKI was responsible for molecular diagnostic testing, was involved in study coordination, data analysis and interpretation and in writing the first draft of the manuscript; ZGM contributed to study design, data analysis and interpretation and preparation of the first draft of the manuscript; AKT was involved in the serological and molecular diagnostic testing; VPK and LGA were involved in the collection and analysis of clinical and epidemiological data; CPM contributed to data interpretation and revised the manuscript; JMH characterised

the B19 viruses, was involved in data analysis and result interpretation as well as the writing of the manuscript.

## References


1. Cossart YE, Field AM, Cant B, Widdows D. Parvovirus-like particles in human sera.Lancet. 1975;305(7898):72-3. DOI: 10.1128/JVI.76.18.9124-9134.2002 PMID: 12186896
2. Servant A, Laperche S, Lallemand F, Marinho V, De Saint Maur G, Meritet JF, et al. Genetic diversity within human erythroviruses: identification of three genotypes. J Virol. 2002;76(18):9124-34. DOI: 10.1128/JVI.76.18.9124-9134.2002 PMID: 12186896
3. Nicolay N, Cotter S. Clinical and epidemiological aspects of parvovirus B19 infections in Ireland, January 1996-June 2008. Euro Surveill. 2009;14(25):19249.PMID: 19555594
4. Young NS, Brown KE. Parvovirus B19.N Engl J Med. 2004;350(6):586-97. DOI: 10.1056/NEJMra030840 PMID: 14762186
5. Toshev A, Ivanova S, Kovaleva V, Andonova L, Mihneva Z. Detection of Human parvovirus B19 (HPVB19) in serum samples from fever-rash ill individuals during the rubella outbreak (2005) in Bulgaria.Biotechnol Biotechnol Equip.2014;28(6):1103-7. DOI: 10.1080/13102818.2014.967746 PMID: 26019596
6. Gonçalves G, Correia AM, Palminha P, Rebelo-Andrade H, Alves A. Outbreaks caused by parvovirus B19 in three Portuguese schools.Euro Surveill. 2005;10(6):121-4.PMID: 16077212
7. World Health Organization (WHO). Strategic plan for measles and congenital rubella infection in the European Region of WHO 2003. Geneva: WHO. Available from: http://www.euro.who.int/__data/assets/pdf_file/0020/79022/E81567.pdf
8. World Health Organization (WHO). Surveillance guidelines for measles, rubella and congenital rubella syndrome in the WHO Europe region. Copenhagen, update December 2012. Geneva: WHO. Available from: http://www.euro.who.int/__data/assets/pdf_file/0018/79020/e93035-2013.pdf?ua=1
9. Siennicka J, Trzcińska A. Comparison of three enzyme immunoassays used to detect human parvovirus B19-specific IgM antibodies in sera of people suspected of measles.Med Sci Monit. 2010;16(5):BR154-9.PMID: 20424545
10. Molenaar-de Backer MW, Lukashov VV, van Binnendijk RS, Boot HJ, Zaaijer HL. Global co-existence of two evolutionary lineages of parvovirus B19 1a, different in genome-wide synonymous positions.PLoS ONE. 2012;7(8):e43206. DOI: 10.1371/journal.pone.0043206 PMID: 22912828
11. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0.Mol Biol Evol. 2007;24(8):1596-9. DOI: 10.1093/molbev/msm092 PMID: 17488738
12. Marinova L, Kojouharova M, Mihneva Z. An ongoing measles outbreak in Bulgaria, 2009.Euro Surveill. 2009;14(26):19259. PMID: 19573512
13. Marinova L, Muscat M, Mihneva Z, Kojouharova M. An update on an ongoing measles outbreak in Bulgaria, April-November 2009.Euro Surveill. 2009;14(50):19442.PMID: 20070938
14. Hübschen JM, Mihneva Z, Mentis AF, Schneider F, Aboudy Y, Grossman Z, et al. Phylogenetic analysis of human parvovirus b19 sequences from eleven different countries confirms the predominance of genotype 1 and suggests the spread of genotype 3b. J Clin Microbiol. 2009;47(11):3735-8. DOI: 10.1128/JCM.01201-09 PMID: 19741071
15. Ramsay M, Reacher M, O'Flynn C, Buttery R, Hadden F, Cohen B, et al. Causes of morbilliform rash in a highly immunised English population. Arch Dis Child. 2002;87(3):202-6. DOI: 10.1136/adc.87.3.202 PMID: 12193426
16. Yermalovich MA, Hübschen JM, Semeiko GV, Samoilovich EO, Muller CP. Human parvovirus B19 surveillance in patients with rash and fever from Belarus.J Med Virol. 2012;84(6):973-8. DOI: 10.1002/jmv.23294 PMID: 22499021
17. Davidkin I, Valle M, Peltola H, Hovi T, Paunio M, Roivainen M, et al. Etiology of measles- and rubella-like illnesses in measles, mumps, and rubella-vaccinated children. J Infect Dis. 1998;178(6):1567-70. DOI: 10.1086/314513 PMID: 9815205
18. Mossong J, Hens N, Friederichs V, Davidkin I, Broman M, Litwinska B, et al. Parvovirus B19 infection in five European countries: seroepidemiology, force of infection and maternal risk of infection. Epidemiol Infect. 2008;136(8):1059-68. DOI: 10.1017/S0950268807009661 PMID: 17956642
19. Yermalovich MA, Semeiko GV, Samoilovich EO, Svirchevskaya EY, Muller CP, Hübschen JM. Etiology of maculopapular rash in measles and rubella suspected patients from Belarus.PLoS ONE. 2014;9(10):e111541. DOI: 10.1371/journal.pone.0111541 PMID: 25356680
20. Handysides S. Parvovirus B19 and intrauterine fetal death – not always associated with hydrops.Euro Surveill. 2001;5(20):1751.
21. Miller E, Fairley CK, Cohen BJ, Seng C. Immediate and long term outcome of human parvovirus B19 infection in pregnancy. Br J Obstet Gynaecol. 1998;105(2):174-8. DOI: 10.1111/j.1471-0528.1998.tb10048.x PMID: 9501782
22. Wermelinger MC, Oelemann WM, Lima de Mendonça MC, Naveca FG, von Hubinger MG. Detection of human parvovirus B19 infection: a study of 212 suspected cases in the state of Rio de Janeiro, Brazil.J Clin Virol. 2002;25(2):223-30. DOI: 10.1016/S1386-6532(02)00014-8 PMID: 12367658
23. Hoebe CJ, Claas EC, Steenbergen JE, Kroes AC. Confirmation of an outbreak of parvovirus B19 in a primary school using IgM ELISA and PCR on thumb prick blood samples.J Clin Virol. 2002;25(3):303-7. DOI: 10.1016/S1386-6532(02)00021-5 PMID: 12423694
24. Corcoran C, Hardie D, Yeats J, Smuts H. Genetic variants of human parvovirus B19 in South Africa: cocirculation of three genotypes and identification of a novel subtype of genotype 1.J Clin Microbiol. 2010;48(1):137-42. DOI: 10.1128/JCM.00610-09 PMID: 19923483
25. Heegaard ED, Panum Jensen I, Christensen J. Novel PCR assay for differential detection and screening of erythrovirus B19 and erythrovirus V9.J Med Virol. 2001;65(2):362-7. DOI: 10.1002/jmv.2042 PMID: 11536245
26. Hokynar K, Norja P, Laitinen H, Palomäki P, Garbarg-Chenon A, Ranki A, et al. Detection and differentiation of human parvovirus variants by commercial quantitative real-time PCR tests. J Clin Microbiol. 2004;42(5):2013-9. DOI: 10.1128/JCM.42.5.2013-2019.2004 PMID: 15131163
27. Cohen BJ, Gandhi J, Clewley JP. Genetic variants of parvovirus B19 identified in the United Kingdom: implications for diagnostic testing.J Clin Virol. 2006;36(2):152-5. DOI: 10.1016/j.jcv.2006.01.011 PMID: 16569510
28. Liefeldt L, Plentz A, Klempa B, Kershaw O, Endres AS, Raab U, et al. Recurrent high level parvovirus B19/genotype 2 viremia in a renal transplant recipient analyzed by real-time PCR for simultaneous detection of genotypes 1 to 3. J Med Virol. 2005;75(1):161-9. DOI: 10.1002/jmv.20251 PMID: 15543575
29. Blümel J, Eis-Hübinger AM, Stühler A, Bönsch C, Gessner M, Löwer J. Characterization of Parvovirus B19 genotype 2 in KU812Ep6 cells.J Virol. 2005;79(22):14197-206. DOI: 10.1128/JVI.79.22.14197-14206.2005 PMID: 16254355
30. Eis-Hübinger AM, Reber U, Edelmann A, Kalus U, Hofmann J. Parvovirus B19 genotype 2 in blood donations.Transfusion. 2014;54(6):1682-4. DOI: 10.1111/trf.12591 PMID: 24911910
31. Grabarczyk P, Kalińska A, Kara M, Wieczorek R, Ejduk A, Sulkowska E, et al. Identification and characterization of acute infection with parvovirus B19 genotype 2 in immunocompromised patients in Poland. J Med Virol. 2011;83(1):142-9. DOI: 10.1002/jmv.21947 PMID: 21108352
32. Norja P, Hokynar K, Aaltonen LM, Chen R, Ranki A, Partio EK, et al. Bioportfolio: lifelong persistence of variant and prototypic erythrovirus DNA genomes in human tissue. Proc Natl Acad Sci USA. 2006;103(19):7450-3. DOI: 10.1073/pnas.0602259103 PMID: 16651522
33. Schneider B, Höne A, Tolba RH, Fischer HP, Blümel J, Eis-Hübinger AM. Simultaneous persistence of multiple genome variants of human parvovirus B19.J Gen Virol. 2008;89(Pt 1):164-76. DOI: 10.1099/vir.0.83053-0 PMID: 18089740
34. Parsyan A, Szmaragd C, Allain JP, Candotti D. Identification and genetic diversity of two human parvovirus B19 genotype 3 subtypes.J Gen Virol. 2007;88(Pt 2):428-31. DOI: 10.1099/vir.0.82496-0 PMID: 17251559
35. Toan NL, Duechting A, Kremsner PG, Song LH, Ebinger M, Aberle S, et al. Phylogenetic analysis of human parvovirus B19, indicating two subgroups of genotype 1 in Vietnamese patients. J Gen Virol. 2006;87(Pt 10):2941-9. DOI: 10.1099/vir.0.82037-0 PMID: 16963753


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# Cluster of atypical adult Guillain-Barré syndrome temporally associated with neurological illness due to EV-D68 in children, South Wales, United Kingdom, October 2015 to January 2016

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**We report a cluster of atypical Guillain-Barré syndrome in 10 adults temporally related to a cluster of four children with acute flaccid paralysis, over a 3-month period in South Wales, United Kingdom. All adult cases were male, aged between 24 and 77 years. Seven had prominent facial diplegia at onset. Available electrophysiological studies showed axonal involvement in five adults. Seven reported various forms of respiratory disease before onset of neurological symptoms. The ages of children ranged from one to 13 years, three of the four were two years old or younger. Enterovirus testing is available for three children; two had evidence of enterovirus D68 infection in stool or respiratory samples. We describe the clinical features, epidemiology and state of current investigations for these unusual clusters of illness.**

## The event

In January 2016, an initial cluster of five cases of Guillain-Barré syndrome (GBS) in adult males was reported to Public Health Wales by neurologists at the University Hospital Wales in Cardiff. Unusually for GBS there was: prominent bilateral facial weakness (facial diplegia), evidence for axonal damage on nerve conduction studies rather than the more typical demyelinating pattern; atypical clinical onset of symptoms and atypical clinical progression. During the initial investigations, two cases of acute flaccid paralysis (AFP) in children from the same areas were identified.

## Background

GBS is an acute inflammatory peripheral nerve disorder of which the commonest type is a demyelinating, ascending paralysis, accounting for around 90% of cases. There are rarer but recognised variants, including Miller Fisher syndrome (MFS), around 5% of GBS, which is typified by the triad of ophthalmoplegia, ataxia and areflexia; and acute motor axonal neuropathy (AMAN), 5% of GBS) in which an axonal rather than demyelinating pattern of nerve damage is seen and tendon reflexes can be paradoxically brisk [1].

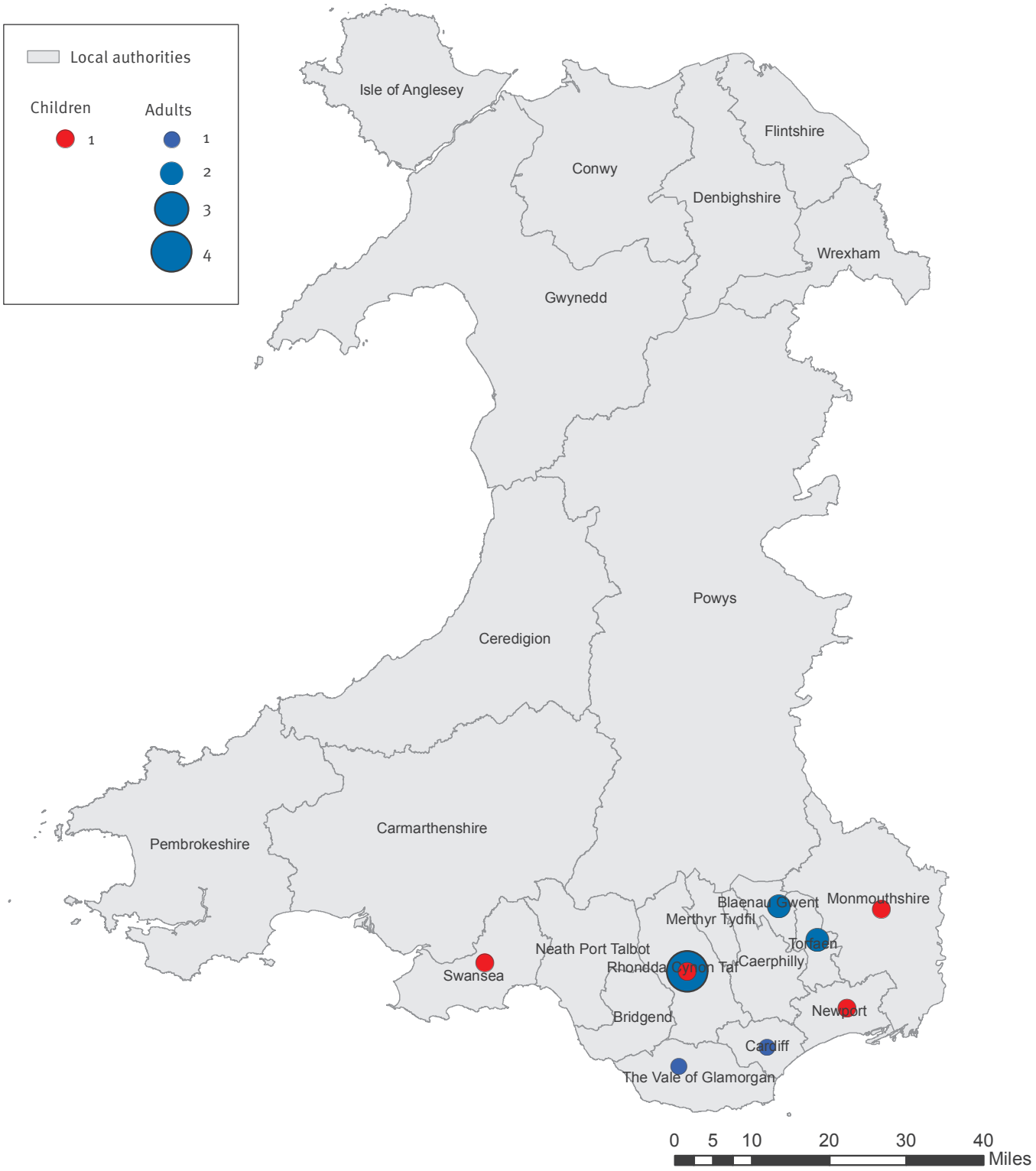
GBS is considered to be an autoimmune disorder triggered by many recognised precipitants via molecular mimicry. There are multiple reports of AMAN clusters, most notably in Asia such as the northeast China outbreaks where an infectious agent has been suspected [2]. AMAN clusters in North America and Europe are much less common and the clinical pattern described by many probable cases here defy contemporary diagnostic categorisation [1].

There are several known infectious triggers for GBS. *Campylobacter* spp. is the most commonly reported prodromal infection, and has been associated with clusters of the axonal variants of GBS in China [3]. Influenza is also a known trigger [4]. More rarely other infectious caused by pathogens such as hepatitis A [5], *Mycoplasma pneumoniae* [6], *Acinetobacter baumannii* [7], cytomegalovirus [8] and Ebstein-Barr virus [9] have been associated with GBS and AMAN syndrome. Recently, hepatitis E virus has been suggested as a possible common trigger for GBS [10], and the emerging Zika and West Nile viruses have also been linked to GBS [11,12].

AFP is a clinical presentation, rather than diagnosis, with rapid onset of weakness of one or more limbs,

**Figure 1**

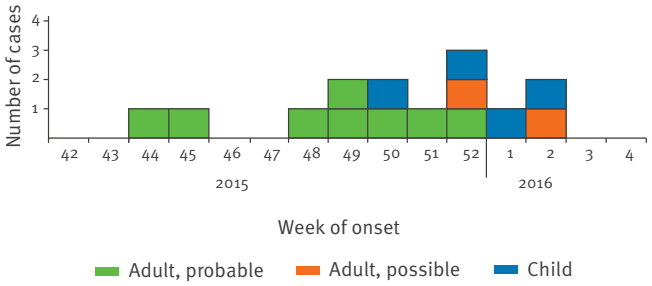
Child and adult cases by local authority, adult cluster of atypical Guillain-Barré syndrome and child cluster of acute flaccid paralysis, South Wales, United Kingdom, October 2015–January 2016 (n = 14)




Source: Public Health Wales. Contains public sector information licensed under the Open Government Licence v2.0 (PSMA: 100004810).

**Figure 2**

Weeks of onset of paralysis for adult and child cases, by case category, adult cluster of atypical Guillain-Barré Syndrome and child cluster of acute flaccid paralysis, South Wales, United Kingdom, October 2015–January 2016 (n = 14)




sometimes associated with weakness of muscles involved in swallowing and respiration, and progression over days and weeks. GBS is one of the differential diagnoses for AFP [13]. Acute poliomyelitis is another, with AFP surveillance an important part of the surveillance for polio. More recently, enterovirus D-68 (EV-D68) has been found to cause an illness presenting with AFP, with radiological evidence of an acute myelitis [14].

The population included in this paper covers the counties of Vale of Glamorgan, Rhondda Cynon Taff, Merthyr Tydfil, Caerphilly, Cardiff, Newport, Blaenau Gwent, Torfaen, Swansea, Bridgend, Neath Port Talbot and Monmouthshire. The population is 1.9 million, with the highest populations found in the coastal city areas of Cardiff (capital of Wales 354,000 inhabitants), Swansea (241,000) and Newport (147,000). The counties of Rhondda Cynon Taff (237,000), Merthyr Tydfil (59,000), Blaenau Gwent (70,000), Torfaen (91,000) and Caerphilly (180,000) cover a hilly area north of the coastal towns, with a strong industrial history but currently relatively high levels of socioeconomic deprivation [15,16]. The area is served by several hospitals and most provide neurology services. Cases requiring electrophysiology, and other specialist care are usually referred to the University Hospital of Wales in Cardiff.

Here we present the investigation of cases and associated results reported as at noon on 20 January 2016.

## Methods

### Case definition

A possible cluster case was defined as a hospitalised patient with either GBS or AFP, in a resident of South Wales, with onset of paralysis on or after 1 September 2015. A probable case was a possible case with investigations showing an axonal neuropathy or with predominant (earlier or more severe) facial weakness or ophthalmoplegia, or a child (under 16 years of age) with AFP. Onset dates refer to the onset of paralysis.

The possible case definition was used to identify cases for further investigation, as both AFP and GBS are rare and investigations required to classify them can take days or weeks to complete.

### Laboratory investigations

Cases were clinically investigated with neuroimaging i.e. magnetic resonance imaging (MRI) and computed tomography (CT) scans, electrophysiological studies (nerve conduction and electromyography), and analysis of anti-ganglioside antibodies as appropriate.

Laboratory investigations included testing of stool, respiratory (throat and naso-pharyngeal swabs), cerebrospinal fluid (CSF) and serum samples for a variety of viral [17] and bacterial pathogens.

All respiratory samples were first screened using a panel of real-time reverse transcription (RT)-PCR assays targeting influenza A and B, respiratory syncytial virus (RSV)-A and -B, human metapneumovirus (hMPV), parainfluenza virus 1-4 and finally a duplex PCR targeting adenovirus and *M. pneumoniae*. A specific rhinovirus assay targeting the 5' untranslated region (UTR) of the *Picornaviridae* family was duplexed with the hMPV assay. To type EV-D68, a further specific assay was used on all samples positive in the EV assay as developed by the EV-D68 European study group [17].

CSF pleocytosis was defined as a white cell count greater than 5 cells per cubic millimetre. Whole blood and urine samples were screened for heavy metals and organophosphate exposure, the latter via a red cell cholinesterase test.

### Epidemiological investigation

Cases were interviewed using a semi-structured questionnaire with open questions to assess recent illnesses, earlier medical history and underlying conditions, as well as environmental and other exposures.

To identify the background incidence of GBS in Wales, we searched routinely collected hospital admission data in Wales with a primary diagnosis of GBS based on the World Health Organization (WHO) International Statistical Classification of Diseases and Related Health Problems (ICD-10) classification G61.0 [18], over a 10-year period between 1 January 2004 and 31 December 2014.

## Results

We identified 10 adults (eight probable, two possible cases) and four children (all probable cases) who met the case definition. Case locations are displayed in Figure 1; most adults lived in local authority areas to the north of the coastal cities in South Wales.

## Table

Demographics, clinical and laboratory findings for cases in adult cluster of atypical Guillain-Barré syndrome and child cluster of acute flaccid paralysis, South Wales, United Kingdom, October 2015-January 2016 (n = 14)

| Case no | Age group (years) | Sex | Neurological diagnosis | Predominant facial/eye symptoms[a] | Nerve conduction studies | Case category | Prodromal illness | CSF pleocytosis[b] | Stool PCR for enterovirus | Respiratory PCR for enterovirus | Respiratory PCR Other pathogens detected |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Adult cluster | | | | | | | | | | | |
| 1 | 40–49 | M | Bifacial weakness | Yes | AMAN | Probable | RTI | No | ND | Neg | None |
| 2 | 50–59 | M | Bifacial weakness / GBS | Yes | AMAN | Probable | RTI | No | Neg | ND | *Haemophilus influenzae*, adenovirus, rhinovirus |
| 3 | 30–39 | M | Bifacial weakness / GBS | Yes | AMSAN | Probable | RTI | ND | ND | ND | Rhinovirus |
| 4 | 60–69 | M | Bifacial weakness / Ophthalmoplegia/ GBS | Yes | AMAN | Probable | RTI | Yes | ND | Neg | Influenza A |
| 5 | 70+ | M | Asymmetric leg weakness | No | AMAN | Probable | None | No | ND | ND | Negative |
| 6 | 40–49 | M | Bifacial weakness / ophthalmoplegia | Yes | ND | Probable | RTI | ND | ND | ND | None |
| 7 | 20–29 | M | Hand predominant GBS | No | AIDP | Possible | RTI | No | Neg | Neg | Adenovirus |
| 8 | 30–39 | M | GBS | No | AIDP | Possible | None | No | ND | ND | None |
| 9 | 40–49 | M | Bifacial weakness / GBS | Yes | ND | Probable | RTI | No | ND | NEG | None |
| 10 | 70+ | M | Bifacial weakness | Yes | ND | Probable | GI | No | ND | ND | None |
| Child cluster | | | | | | | | | | | |
| 1 | 0–4 | F | AFP | No | AMAN | Probable | RTI | Yes | EV-D68 | Neg | None |
| 2 | 0–4 | M | Lower limb paralysis | No | ND | Probable | RTI | Yes | ECHO25 | Neg | None |
| 3 | 10–14 | F | GBS | No | AMSAN | Probable | RTI | No | EV-D68 | EV-D68 | None |
| 4 | 0–4 | F | Bifacial weakness / ophthalmoplegia | Yes | ND | Probable | None | No | Neg | ND | None |

AFP: acute flaccid paralysis; AMAN: acute motor axonal neuropathy; AMSAN: acute motor sensory axonal neuropathy; CSF: cerebrospinal fluid; ECHO 25: echovirus 25; EV: enterovirus; F: female: GBS: Guillain-Barré Syndrome; GI: gastrointestinal illness; M: male; Neg: negative; ND: not done; Pos: positive; RTI: respiratory tract infection.

[a]These are cases where facial weakness or ophthalmoplegia were present early in the illness, or were more severe than limb weakness.

[b]CSF pleocytosis was defined as more than 5 white cells per cubic millimetre.

Cases of enterovirus and echovirus 25 in children are highlighted in grey.`

All adult cases were male, with a median age of 45 years (range 24–77). The dates of onset of paralysis ranged from 29 October 2015 to 8 January 2016 (Figure 2).

Eight of the 10 adult cases had a prodromal illness consisting of respiratory tract manifestations including sore throats, lower and other upper respiratory tract symptoms and ear infections in seven, and diarrhoeal illness in one.

The four child cases all presented with AFP; one had prominent facial and eye symptoms. They were three females and one male, aged between 1 and 13 years, with three aged 2 years or younger. Three of four had a respiratory infection preceding onset of AFP. They resided in four local authority areas (Figure 1).

The Table summarises the clinical and microbiological features of the adult and child cases.

## Neurological findings

Of the eight probable adult cases, seven presented with prominent, mainly asymmetric facial weakness. One (Case 5) had predominantly lower limb weakness and an AMAN pattern on electrophysiology. In five of the eight cases nerve conduction studies were typical for AMAN or AMSAN. Case 5 was the only immunosuppressed individual (taking 50mg oral prednisolone per day); he presented with an acute asymmetric lower limb paralysis and electrophysiologically diagnosis was AMAN. Ophthalmoplegia was only a feature in one possible case who had a demyelinating pattern on electrophysiology.

The possible Cases 7 and 8 were more consistent with classical GBS, presenting with ascending or peripheral

weakness; both had electrophysiology consistent with Acute Inflammatory Demyelinating Polyneuropathy (AIDP).

All eight probable cases received immunoglobulin and seven of them had either a good or excellent response to it. Two cases (Cases 2 and 6) had positive titres to anti-GQ1b antibodies.

Three adults had spinal MRIs (Cases 10, 11 and 12), with no abnormalities detected; five had a cranial CT or MRI, with no evidence of a cause for the acute illness.

Three of the four children presented with an AFP; one of them with marked weakness of lower limbs only. The other child presented with an AFP with ophthalmoplegia and prominent facial weakness. Two had evidence of signal change on cervical spine MRI in keeping with a transverse myelitis (Cases 1 and 2).

## Microbiological and toxicological investigations

Only one adult had a CSF leucocytosis (WBC 53); this case did not meet the criteria for a probable case. The clinical picture involved a complex and progressive ophthalmoplegia and asymmetric facial diplegia and the putative diagnosis of Bickerstaff's encephalitis was made. Results of PCR tests on respiratory samples from all 10 adult cases were positive for rhinovirus or adenovirus in two cases, respectively, one sample was positive for influenza and *Haemophilus influenzae* was identified by sputum culture in one case, which also tested positive for rhinovirus and adenovirus (PCR, included in above results). Two adults were tested for hepatitis B and C and one for hepatitis E; all were negative. Stool culture was performed in three of the adult cases and was negative in all of them.

Two children had white cells in their CSF, one was predominantly lymphocytic. Three of the four children had real-time RT-PCR evidence of EV in stool samples, of which two were typed as EV-D68 and the third had an echovirus 25; one child who was EV-D68-positive in stool also had a respiratory sample positive for EV-D68. One also had evidence of infection with *M. pneumoniae* by respiratory real-time RT-PCR and serology.

Biochemistry results were reported for four adult cases. Tests for heavy metals in whole blood confirmed no abnormalities in all of them. Six adults were tested for organophosphate exposure: one showed no abnormalities and five are still awaiting results.

## Environmental exposures

All 10 adult cases were interviewed. Exposures reported more than once included contact with dogs (4/10) and other pets (2/10); tattoos (4/10); smoking (2/10, of those 1 e-cigarettes) and clay pigeon shooting (2/10). Two had travelled outside the United Kingdom (UK) in the two months before onset of illness to Spain and Bulgaria, respectively. None had travelled to an area reported to have had cases of Zika virus. None had had any recent vaccinations, including seasonal influenza vaccine, and none reported use of illicit substances, significant changes in medication or use of alternative medicines.

The four child cases were all age-appropriately vaccinated and none had had recent medication changes or exposure to toxic household chemicals; three had exposure to dogs or cats, and one had household exposure to a smoker. None had travelled outside the UK in the two months before onset of illness.

## Excluded case

In addition to the four cases presented above, a further child in Wales had AFP with ophthalmoplegia and evidence of EV-D68 in stool samples during the same period, and was investigated as part of the cluster. However, they were excluded as they fell outside the area included in the initial case definition.

## Hospital admissions data 2004–14

There were an average of 69 (range 49–91) admissions with GBS per year, an average of six (range 0–13) admissions per month. Admissions showed a seasonal pattern with the greatest number of admissions occurring in January (data not shown).

The background incidence of GBS in Europe is 1.2 to 1.9 per 100,000 [19], which would equate to around 36 to 58 cases per year, or three to five cases per month for all Wales (population 3.07 million [16]).

# Discussion

The adult and child cases presented are clustered in both space and time, with adult cases showing predominantly the more unusual variant forms of GBS. The majority of adult cases showed AMAN and AMSAN i.e. features of the axonal variants of GBS. These variants are rare in Europe and the United States, where they account for only 5% of GBS cases. However, they are more common in China, Japan and Central and South America where they constitute 30–47% of cases [19]. None of our cases meet the diagnostic criteria for MFS [1], which has a much lower incidence than GBS (0.1/100,000 compared with 1.2–1.9/100,000 [19]).

This cluster of 10 adult cases over a three-month period would constitute a winter peak within the context of population estimates and hospital episode data for the past decade. What is unusual, compared with expected winter peaks of incidence, are the clinical features of facial weakness and asymmetric onset, and electrophysiological features of axonal involvement. The strong male predominance (all adults were male) is also unusual, with the usual male:female ratio estimated at 1.5:1 [19].

Both adult and child cases cluster geographically outside major cities, with 12 of 14 living outside the three main cities in the area (Cardiff, Newport and Swansea),

despite 48% of the population of the counties with cases being in these cities [16].

The child cases in our cluster are clinically different from the cases in the adult cluster, presenting with a syndrome similar to that previously reported for EV-D68 associated AFP, with half having radiological evidence of a transverse myelitis. Adult AFP cases have been reported coincident with a rise in EV-D68 cases [14], but in this series child cases predominated, with only nine of 59 cases aged over 21 years. Twelve children in Colorado had a neurological illness at a median of seven days following a febrile infection, with EV-D68 detected in five of 11 tested [20]. Their symptoms were flaccid limb weakness in 10 cases, bulbar weakness in six and two had facial weakness, accompanied by spinal MRI changes.

There is no clear hypothesis as yet for the cause of the adult cluster. The temporal association of the two clusters could represent a coincidence, or an artefact due to changes in local diagnosis. Current hypotheses under investigation include enterovirus infection, either EV-D68 or other types; gastrointestinal infections such as *Campylobacter* spp., hepatitis E infection and influenza. Variant forms of GBS have been associated with *C. jejuni* [3], but here there is no stool culture evidence of campylobacteriosis. Emerging arbovirus infections such as Zika and West Nile virus are unlikely as causes for the neurological symptoms due to the absence of suitable vectors in Wales and any appropriate travel history. The clinical picture of cases was not consistent with botulism.

Descriptive epidemiology, with predominance of adult males and some geographic clustering, might suggest an environmental or behavioural exposure. However, no common exposure supporting this theory has been identified and no evidence of toxic causes has been found from clinical samples so far.

All but four of the cases had a preceding respiratory tract infection. Influenza was first reported as circulating in Wales from week 1 in 2016 [21], post-dating the illness for these cases. Respiratory samples are not routinely tested for enterovirus, but retrospective PCR testing of respiratory samples from children (under 16 years of age) taken from 1 December to 6 January, found 17 of 163 with evidence of enterovirus and five of 17 with EV-D68.

The methods used in defining the clusters have limitations, mainly in keeping with an evolving early report. Not all cases have undergone the same level of neuroimaging, electrophysiological and microbiological testing, so the cases are defined mainly by their clinical presentation. Exposures have not yet been systematically obtained using a standardised, closed-question questionnaire. A further review, including more intensive case finding, is being undertaken to better characterise the cluster.

The child cases result either from increased recognition and diagnosis or increased true incidence of neurological disease caused by infection with EV-D68. Neurological illness due to EV-D68 has not previously been described in Wales. The adult and child clusters may be completely separate, but are presented together here because of their temporal association, and because EV-D68 neurological illness is a relatively rare diagnosis in the UK, although EV-D68 is thought likely to be circulating in the community [22].

Further investigations planned include serology for hepatitis E, influenza and other possible infection triggers, using stored serum and new samples to be taken after the intravenous immunoglobulins have left the patients' serum. Further antiganglioside antibody testing, and human leukocyte antigen (HLA) typing are also planned. Surveillance for atypical GBS continues in Wales using a standardised reporting form and following case finding alerts to clinicians, and enhanced surveillance for enterovirus infection is also planned. Public Health Wales has alerted clinicians, and has been working with Public Health England and the European Centre for Disease Prevention and Control (ECDC) to inform other European Union countries about this cluster via the Early Warning and Response System (EWRS).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## Conflict of interest

None declared.

## Authors' contributions

RHT and TPP: Identifying cases, clinical description of cases;

CJW: Descriptive epidemiology, background and epidemiological methods;

GL: Principal investigator for the clusters;

ML: Managerial oversight and hypothesis generation;

HB: Biological sampling of environmental hazard exposures – methods and results;

RhES: Case exposure history ascertainment, questionnaire data collation, laboratory, clinical and toxicology liaison;

BWM: Conceived need for work, critically revised content for important intellectual content, approved final version;

CM, RJ, RH: Microbiological advice and specialist testing;

AA: Article conception as part of communications strategy.

## References


1. Wakerley BR, Uncini A, Yuki N, Attarian S, Barreira AA, Chan Y-C, et al. Guillain-Barré and Miller Fisher syndromes--new diagnostic classification. Nat Rev Neurol. 2014;10(9):537-44. DOI: 10.1038/nrneurol.2014.138 PMID: 25072194
2. Ye Y, Zhu D, Wang K, Wu J, Feng J, Ma D, et al. Clinical and electrophysiological features of the 2007 Guillain-Barré syndrome epidemic in northeast China. Muscle Nerve. 2010;42(3):311-4. DOI: 10.1002/mus.21701 PMID: 20589890
3. Ho TW, Mishu B, Li CY, Gao CY, Cornblath DR, Griffin JW, et al. Guillain-Barré syndrome in northern China. Relationship to Campylobacter jejuni infection and anti-glycolipid antibodies. Brain. 1995;118(Pt 3):597-605. DOI: 10.1093/brain/118.3.597 PMID: 7600081
4. Kutleša M, Santini M, Krajinović V, Raffanelli D, Barsić B. Acute motor axonal neuropathy associated with pandemic H1N1 influenza A infection.Neurocrit Care. 2010;13(1):98-100. DOI: 10.1007/s12028-010-9365-y PMID: 20428968
5. Chitambar SD, Fadnis RS, Joshi MS, Habbu A, Bhatia SG. Case report: Hepatitis A preceding Guillain-Barré syndrome.J Med Virol. 2006;78(8):1011-4. DOI: 10.1002/jmv.20656 PMID: 16789022
6. Susuki K, Odaka M, Mori M, Hirata K, Yuki N. Acute motor axonal neuropathy after Mycoplasma infection: Evidence of molecular mimicry.Neurology. 2004;62(6):949-56. DOI: 10.1212/01.WNL.0000115123.42929.FD PMID: 15037698
7. Toscani L, Guarducci D, Matà S, Furlan T, Ballo P. Association between acute motor axonal neuropathy and septic shock due to Acinetobacter baumannii.Infez Med. 2015;23(4):349-52. PMID: 26700086
8. Orlikowski D, Porcher R, Sivadon-Tardy V, Quincampoix JC, Raphaël JC, Durand MC, et al. Guillain-Barré syndrome following primary cytomegalovirus infection: a prospective cohort study. Clin Infect Dis. 2011;52(7):837-44. DOI: 10.1093/cid/ciro74 PMID: 21427390
9. Masajtis-Zagajewska A, Muras K, Mochecka-Thoelke A, Kurnatowska I, Nowicki M. Guillain-Barré syndrome in the course of EBV infection after kidney transplantation--a case report.Ann Transplant. 2012;17(3):133-7. DOI: 10.12659/AOT.883468 PMID: 23018266
10. Dalton HR, Kamar N, van Eijk JJ, Mclean BN, Cintas P, Bendall RP, et al. Hepatitis E virus and neurological injury. Nat Rev Neurol. 2015.
11. Oehler E, Watrin L, Larre P, Leparc-Goffart I, Lastere S, Valour F, et al. Zika virus infection complicated by Guillain-Barre syndrome--case report, French Polynesia, December 2013. Euro Surveill. 2014;19(9):20720. DOI: 10.2807/1560-7917.ES2014.19.9.20720 PMID: 24626205
12. Sejvar JJ, Bode AV, Marfin AA, Campbell GL, Ewing D, Mazowiecki M, et al. West Nile virus-associated flaccid paralysis. Emerg Infect Dis. 2005;11(7):1021-7. DOI: 10.3201/eid1107.040991 PMID: 16022775
13. Marx A, Glass JD, Sutter RW. Differential diagnosis of acute flaccid paralysis and its role in poliomyelitis surveillance. Epidemiol Rev. 2000;22(2):298-316. DOI: 10.1093/oxfordjournals.epirev.a018041 PMID: 11218380
14. Van Haren K, Ayscue P, Waubant E, Clayton A, Sheriff H, Yagi S, et al. Acute Flaccid Myelitis of Unknown Etiology in California, 2012-2015. JAMA. 2015;314(24):2663-71. DOI: 10.1001/jama.2015.17275 PMID: 26720027
15. Welsh Government. Chief Medical Officer for Wales. Annual Report 2014-15. Cardiff: Welsh Government. 2015. Available from: http://gov.wales/docs/phhs/publications/151014cmoreporten.pdf
16. Welsh Government. StatsWales. 2014 mid-year population estimates. Cardiff: Welsh Government. [Accessed: 25 Jan 2016]. Available from: https://statswales.wales.gov.uk/Catalogue
17. Poelman R, Schuffenecker I, Van Leer-Buter C, Josset L, Niesters HG, Lina B,, et al. European surveillance for enterovirus D68 during the emerging North-American outbreak in 2014. J Clin Virol. 2015;71:1-9. DOI: 10.1016/j.jcv.2015.07.296 PMID: 26364237
18. World Health Organization (WHO). International Statistical Classification of Diseases and Related Health Problems. 10th Revision (ICD-10). Geneva: WHO. [Accessed 25 Jan 2016]. Available from: http://www.who.int/classifications/icd/ICD10Volume2_en_2010.pdf
19. Hughes RAC, Cornblath DR. Guillain-Barré syndrome.Lancet. 2005;366(9497):1653-66. DOI: 10.1016/S0140-6736(05)67665-9 PMID: 16271648
20. Messacar K, Schreiner TL, Maloney JA, Wallace A, Ludke J, Oberste MS, et al. A cluster of acute flaccid paralysis and cranial nerve dysfunction temporally associated with an outbreak of enterovirus D68 in children in Colorado, USA. Lancet. 2015;385(9978):1662-71. DOI: 10.1016/S0140-6736(14)62457-0 PMID: 25638662
21. Public Health Wales. Weekly influenza activity in Wales report. Cardiff: Public Health Wales. 20 Jan 2016. Available from http://www.wales.nhs.uk/sites3/page.cfm?orgid=457&pid=34338
22. Public Health England (PHE). Risk assessment of Enterovirus D-68 (EV-D68). London: PHE. Jan 2015. Available from www.gov.uk/government/uploads/system/uploads/attachment_data/file/399590/EV-D68_RA_v4__3_.pdf


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# Systematic review on tuberculosis transmission on aircraft and update of the European Centre for Disease Prevention and Control risk assessment guidelines for tuberculosis transmitted on aircraft (RAGIDA-TB)

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**As a setting for potential tuberculosis (TB) transmission and contact tracing, aircraft pose specific challenges. Evidence-based guidelines are needed to support the related-risk assessment and contact-tracing efforts. In this study evidence of TB transmission on aircraft was identified to update the Risk Assessment Guidelines for TB Transmitted on Aircraft (RAGIDA-TB) of the European Centre for Disease Prevention and Control (ECDC). Electronic searches were undertaken from Medline (Pubmed), Embase and Cochrane Library until 19 July 2013. Eligible records were identified by a two-stage screening process and data on flight and index case characteristics as well as contact tracing strategies extracted. The systematic literature review retrieved 21 records. Ten of these records were available only after the previous version of the RAGIDA guidelines (2009) and World Health Organization guidelines on TB and air travel (2008) were published. Seven of the 21 records presented some evidence of possible in-flight transmission, but only one record provided substantial evidence of TB transmission on an aircraft. The data indicate that overall risk of TB transmission on aircraft is very low. The updated ECDC guidelines for TB transmission on aircraft have global implications due to inevitable need for international collaboration in contract tracing and risk assessment.**

## Background

Air travel has greatly increased in recent decades [1]. To guide countries and harmonise actions in case of potential tuberculosis (TB) transmission on an aircraft, the World Health Organization (WHO) published a first edition of guidelines on TB prevention and control in regards to air travel in 1998, which recommended informing passengers of the exposure with appropriate advice on follow-up. In 2006 and 2008 [2], updates that recommended more extensive screening of in-flight contacts of infectious TB patients followed the first edition. These changes were influenced by specific incidents. For example, in 2007, notable media attention was attracted by a case of a multidrug-resistant (MDR-) TB patient who flew on two long-haul flights [3-7]. In 2009 the European Centre for Disease Prevention and Control (ECDC) published their Risk Assessment Guidelines for Infectious Diseases Transmitted on Aircraft (RAGIDA) [8], where TB was included among 11 other communicable diseases. Compared with the WHO guidelines, RAGIDA-TB limited the extent of investigations. A subsequent systematic review found limited evidence of TB transmission and further challenged the premise for more intense contact investigation [9]. In 2013, ECDC conducted a stakeholder survey to assess the current overall RAGIDA guidelines in order to guide their further development. Based on the replies, a process to update several chapters of the guidelines, including the RAGIDA-TB chapter, was initiated [10]. This paper presents the results of the systematic literature review conducted to update the evidence base on the risk of TB transmission during air travel. It summarises the ECDC recommendations and discusses the major differences compared with other widely used TB and air travel guidelines.

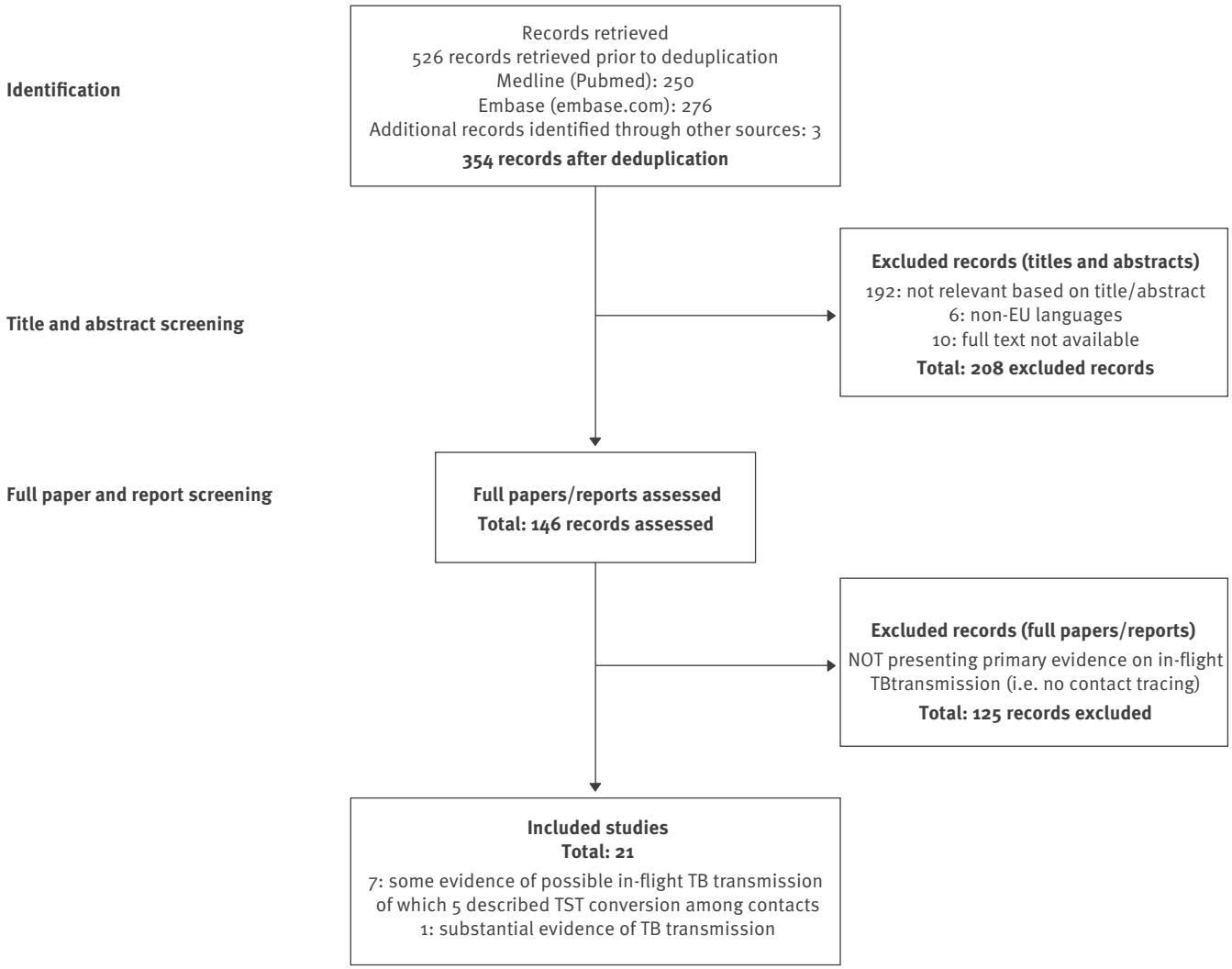
## Methods

### Literature search

Electronic searches identified primary evidence on TB transmission on aircraft from Medline (Pubmed) and Embase up to 19 July 2013. A general search of Cochrane Library identified relevant systematic reviews. No

**Figure**

Risk Assessment Guidelines for Infectious Diseases Transmitted on Aircraft (RAGIDA) tuberculosis literature search: study selection




language or date restrictions were applied. The search strategies are presented in the Box.

The titles and abstracts of all identified hits were filtered by two reviewers. Only human exposures in aircraft settings were retained. For records lacking abstracts, the full text of records with relevant titles was considered. Consensus between the two reviewers was reached on the records to be retained in the analysis. Subsequently, full texts of those abstracts chosen were evaluated in depth by one reviewer for primary evidence on TB transmission on aircraft. Additional records missed by the searches were detected in the lists of references of relevant records. The data extracted were: flight characteristics, such as origin, destination and type of aircraft, year of flight, total in-flight time including ground delay, total number of passengers; characteristics of the index cases such as age, sex, symptoms before and during the flight and at diagnosis, infectiousness, resistance profile of the isolate, and seating characteristics; country initiating passenger contact tracing, time period and strategy of contact tracing, total number of contacts and successfully traced contacts as well as contacts with positive test results and test converters. Records in non-European Union (EU) languages were excluded.

A possible event of in-flight transmission of TB was defined as: tuberculin skin test (TST) conversion (negative baseline result and a subsequent positive result eight weeks or more after exposure) or positive test for TB infection (TST or interferon-gamma release assay (IGRA)) with no other known previous TB exposure or risk factors for a positive test (such as Bacillus Calmette–Guérin (BCG) vaccination), diagnosed during a contact investigation eight weeks or more after TB exposure on an aircraft. The risk of transmission was estimated by calculating the proportions of converters and test-positive contacts (including the converters) without other risk factors among all tested passenger contacts. We calculated the proportion as indicator of transmission risk separately for incidents where the

**Box**

European Centre for Disease Prevention and Control (ECDC) risk assessment guidelines for tuberculosis transmitted on aircraft: literature search strategies, July 2013

**Embase (embase.com)**

#1 'aerospace medicine'/exp OR 'aircraft'/exp OR 'flight'/exp OR 'airplane crew'/exp OR 'airplane pilot'/exp OR 'aviation'/exp OR 'aero transport':ab,ti OR aircraft*:ab,ti OR aeroplane*:ab,ti OR airline*:ab,ti OR airplane*:ab,ti OR flight*:ab,ti OR aircrew:ab,ti OR airflight*:ab,ti OR aviation:ab,ti OR airport*:ti,ab OR aeroport*:ti,ab OR 'air port':ti,ab OR steward:ti,ab OR stewardess:ti,ab OR inflight:ti,ab OR 'in-flight':ti,ab OR 'cabin crew':ti,ab OR cabin:ti,ab OR cabins:ti,ab OR 'air-travel':ab,ti OR ((travel* OR transport* OR journey* OR trip OR trips) NEAR/4 air):ab,ti OR 'air-transport':ti,ab OR ((plane OR planes) AND (air OR travel* OR transport* OR journey* OR trip OR trips)):ti,ab OR ((passenger* OR crew OR traveller* OR personnel OR staff) NEAR/4 (flying OR air OR fly)):ab,ti

#2 'tuberculosis'/exp OR 'mycobacterium tuberculosis'/exp OR tb:ab,ti OR tuberculosis:ab,ti OR tuberculoses:ab,ti OR mtb:ab,ti OR tuberculous:ab,ti

#3 ('time-of- flight' AND spectrometry):ti,ab

#4 #1 AND #2

#5 #4 NOT #3

Limits: no limits

Results: 250

**Medline (Pubmed)**

#1 "Aerospace Medicine"[Mesh] OR "Aircraft"[Mesh] OR "Aviation"[Mesh] OR "Airports"[Mesh] OR aircraft*[tiab] OR aeroplane*[tiab] OR airline*[tiab] OR flight*[tiab] OR aircrew[tiab] OR airflight*[tiab] OR airplane*[tiab] OR aviation[tiab] OR airport*[tiab] OR aeroport*[tiab] OR "aero transport"[tiab] OR "air port"[tiab] OR steward[tiab] OR stewardess[tiab] OR inflight[tiab] OR "in-flight"[tiab] OR "cabin crew"[tiab] OR cabin[tiab] OR cabins[tiab] OR ((travel*[tiab] OR "Travel"[Mesh] OR transport*[tiab] OR journey*[tiab] OR trip[tiab] OR trips[tiab]) AND air[tiab]) OR ((plane[tiab] OR planes[tiab]) AND (air[tiab] OR travel*[tiab] OR "Travel"[Mesh] OR transport*[tiab] OR journey*[tiab] OR trip[tiab] OR trips[tiab])) OR ((passenger*[tiab] OR crew[tiab] OR traveller*[tiab] OR personnel[tiab] OR staff[tiab]) AND (flying[tiab] OR fly[tiab] OR air[tiab]))

#2 "Tuberculosis"[Mesh] OR "Mycobacterium tuberculosis"[Mesh] OR tb[tiab] OR tuberculosis[tiab] OR tuberculosis[tiab] OR mtb[tiab] OR tuberculous[tiab]

#3 ("time of flight"[tiab] AND spectrometry[tiab])

#4 #1 AND #2

#5 #4 NOT #3

Limits: no limits

Results: 276

Results without duplicates: 351

Date of searches 19 July 2013

contact-tracing strategy included all passengers and for cases where only five rows surrounding the index case were traced.

### RAGIDA-TB update 2014

The relevant publications served as an evidence base for the RAGIDA-TB update by an expert group during a meeting in Stockholm in October 2013, coordinated by the ECDC. The data extracted during the systematic literature review were peer-reviewed by the expert group. The guidance document was finalised by the experts in the first quarter of 2014.

All decisions of the expert group were evaluated using GRADE criteria [11], considering: (i) quality of evidence; (ii) the balance between desirable and undesirable effects (whether the benefits are directed to the right group, i.e. the passengers suspected of having contracted TB infection); (iii) uncertainty or variability in values and preferences, i.e. whether the individuals (contacts) are willing to be screened for TB, and (iv) whether the intervention represents a wise use of resources.

## Results

### Literature search

The literature search retrieved 354 unique hits (Figure ).

During the abstract screening stage 208 records were excluded (six based on title and keywords only). Ten records were discarded because their full texts were no longer available (nine were published in the 1950s or before, and one was published in 1995 but was not available from the publisher). At the full text screening stage, 125 records were discarded where the setting was not aircraft and/or population not human, i.e. not presenting data on contact tracing after in-flight exposure. Finally, 21 records (of which three were unindexed records that were detected by browsing in the lists of references of the records identified in the literature search) were retained [3,4,12-30]. Within these 21 records, 27 flights were described where contact tracing was initiated following a potential TB transmission from a passenger, and three records presented aggregated data from the United States, Canada and the United Kingdom (UK) on 252 flights [28-30]. Furthermore, three incidents where the index case was a crew member were described [15,23,24]. Ten of the 21 records [3,4,12,17-19,24,28-30] had not been included in the 2009 version of RAGIDA-TB [8]. A summary of the extracted data is presented in Table 1.

In 14 of the 21 studies, no evidence of in-flight TB transmission was identified. Seven of the 21 studies [15,16,21,24-26,29] presented some evidence of possible in-flight transmission. All flights had lasted more than eight hours. Five of these articles [15,16,21,26,29] described TST conversion among contacts.

**Table 1 a**

Summary of evidence on tuberculosis (TB) transmission on aircraft, systematic review 2013 (n=21 studies)

| Study | Number of flights | Flight duration (h) | Contact tracing strategy | Infectiousness during the flight | Resistance profile of isolate | Total number of aircraft contacts | Number of aircraft contacts tested/ results available (% of all contacts) | Positive contacts/ converters | Converters | Positive contacts including converters/ converters possibly infected during the flight, with no other risk factors for TB infection positivity | Other information on positive contacts possibly infected during the flight |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Kim 2012 [17] | 1 | >8 | 5 rows | Smear-positive, cavitary disease | Not known | 15 | 2 (13) | 0 | 0 | No | No |
| Thibeault 2012 [24] | Crew member flying for 1 month | NA | Crew | Smear-positive, cavitary disease | Not known | 56 | 32 (57) | 6 | Not known | 6/0 | 6 TST-positive crew members of whom 4 tested with IGRA ⇢ 1 positive Country of origin not known Age: two contacts<45 y, 4 contacts 45 y or older |
| Lynggaard 2011 [19] | 1 | 12 | 5 rows | Smear-positive | DS-TB | 28 | 22 (79) | 1 | 0 | No | No |
| Scholten 2010 [30] | 109[a] | Aggregated data | 5 rows | Culture-positive | Aggregated data | Not known | Not known | Not known | Not known | No | No |
| Marienau 2010 [29] | 131 | ≥8 | 5 rows | Pulmonary/ laryngeal TB without adequate treatment | Aggregated data | 4,638 | 758 (16) | 182 | 8 | 12/1 | Passengers seated in the same row or within 2 rows Not originating from a high TB prevalence country, ages not known |
| Kornylo-Duong 2009 (1) [18] | 2 | 14/15 | 5 rows | Smear-positive, cavitary disease | MDR-TB | 79 | 45 (57) | 16 | 3 | No | No |
| Kornylo-Duong 2009 (2) [18] | 2 | 7.5 | 5 rows | Smear-positive, cavitary disease | DS-TB | 78 | 26 (33) | 0 | 0 | No | No |
| Chemardin 2007 [14] | 1 | 5 | 5 rows | Smear-positive, severe cough | XDR-TB | 11 | 3 (27) | 0 | 0 | No | No |
| Abubakar 2008 [28] | 11 | ≥8 | 5 rows | Aggregated data | Aggregated data | Not known | 2 (not known) | 0 | 0 | No | No |
| Buff 2008/ECDC 2007 [3,4] | 2 | >8 | 5 rows | Smear-negative | MDR-TB | 72 | 54 (75) | 21 | 0 | No | No |

DS: drug-susceptible; IGRA: interferon-gamma release assay; MDR: multidrug-resistant; NA: not applicable; TB: tuberculosis; TST: tuberculin skin testing; US: United States; y: years; XDR: extensively drug-resistant.

[a]110 in the article, but one of the incidents has been published separately [3,4].

**Table 1 b**

Summary of evidence on tuberculosis (TB) transmission on aircraft, systematic review 2013 (n=21 studies)

| Study | Number of flights | Flight duration (h) | Contact tracing strategy | Infectiousness during the flight | Resistance profile of isolate | Total number of aircraft contacts | Number of aircraft contacts tested/ results available (% of all contacts) | Positive contacts/ converters | Converters | Positive contacts including converters/ converters possibly infected during the flight, with no other risk factors for TB infection positivity | Other information on positive contacts possibly infected during the flight |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Whitlock 2001 [27] | 2 | 8 | Same cabin section/all passengers and crew | Smear-positive, cavitary disease | DS-TB | 238 | 142 (60) | 24 | 4 | No | No |
| Wang 2000 [26] | 1 | 14 | All passengers and crew (Taiwan residents) | Smear-positive, cavitary disease | Not known | 308 | 212 (69) | 193 | 9 | 3/3 | Passengers seated at a distance of at least 12 rows from the index case<br>Resided in Taiwan, ages 55–57 years |
| Parmet 1999 [23] | Crew member, contact exposure time 8–60 hours | NA | Crew | Not known | Not known | 48 | 38 (79) | 0 | 0 | No | No |
| Vassiloyanakopoulos 1999 [25] | 1 | >8 | All passengers and crew | Smear-positive | Monoresistant TB | 148 | 7 (5) | 1 | 0 | 1/0 | Passenger with positive baseline test<12 weeks after exposure, exact time of testing or seating in relation to index case not known |
| Beller 1996 [12] | 1 | 2.5 | All passengers and crew | Smear-positive | DS-TB | 12 | 9 (75) | 0 | 0 | No | No |
| Kenyon 1996 [16] | 4 | 8/2/2/9 | All passengers and crew with known US or Canadian residence | Smear-positive, cavitary disease | MDR-TB | 1,042 | 760 (73) | 29 | 6 | 6/4 | 2 converters and 2 positive contacts seated within two rows<br>Countries of origin not known<br>Ages 36–55 years |

DS: drug-susceptible; IGRA: interferon-gamma release assay; MDR: multidrug-resistant; NA: not applicable; TB: tuberculosis; TST: tuberculin skin testing; US: United States; y: years; XDR: extensively drug-resistant.

## Table 1 c

Summary of evidence on tuberculosis (TB) transmission on aircraft, systematic review 2013 (n=21 studies)

| Study | Number of flights | Flight duration (h) | Contact tracing strategy | Infectiousness during the flight | Resistance profile of isolate | Total number of aircraft contacts | Number of aircraft contacts tested/ results available (% of all contacts) | Positive contacts/ converters | Converters | Positive contacts including converters/ converters possibly infected during the flight, with no other risk factors for TB infection positivity | Other information on positive contacts possibly infected during the flight |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Miller 1996 [21] | 2 | 8.5/1.5 | All passengers and crew | Smear-positive | DS-TB | 219 | 120 (55) | 34 | 5 | No | All positive passenger contacts had other risk factors, but TST positivity was associated with sitting within 1 row. |
| Moore 1996 [22] | 2 | 1 | All passengers and crew with known US residence | Smear-positive, cavitary and laryngeal disease | DS-TB | 227 | 100 (44) | 5 | Not known | No | No |
| CDC 1995 [13] | 5 | 0.5/3/9/3/0.5 | All passengers with US residence | Smear-positive, cavitary disease | Not known | 753 | 109 (14) | 24 | Not known | No | No |
| Driver 1994 [15] | Crew member flying during 6 months | NA | Crew members and frequent flyers who flew with the index case | Smear-positive, cavitary disease | DS-TB | 345 | 271 (79) | 27 | 2 | 6/2 | 2 crew members converted, 4 frequent flyers positive. Crew members both from US, ages not known Passengers from Northern America, ages not known |
| McFarland 1993 [20] | 1 | 8 | All US citizens (passengers and crew) | Smear-positive, cavitary disease | MDR-TB | 343 | 79 (23) | 8 | Not known | No | NA |
| **Total** | **279** | **NA** | **NA** | **NA** | **NA** | **8660** | **2791** | **571** | **37** | **34/10** | **NA** |

DS: drug-susceptible; IGRA: interferon-gamma release assay; MDR: multidrug-resistant; NA: not applicable; TB: tuberculosis; TST: tuberculin skin testing; US: United States; y: years; XDR: extensively drug-resistant.

In two of the studies [15,24] the index case and the contacts positive for TB infection were crew members, and it was not possible to exclude transmission on the ground (before and after the flight when the aircraft ventilation system is not in full-function mode as well as outside the aircraft). However, in one of these papers [15] TB transmission from the index case to passengers was implied. In five other studies [16,21,25,26,29] with possible TB transmission, the index case was a smear-positive passenger (i.e. sputum sample positive for acid-fast bacilli in microscopic examination). In the study by Wang et al. [26], three converters with no prior TB exposure or BCG vaccination were found among 212 passenger contacts. However, all of them had been seated at least 15 rows away from the index case and an in-flight transmission does not seem probable. Vassiloyanakopoulos et al. [25] found one passenger contact with a positive TST, but the infection could have been acquired before the flight. The study from Marienau et al. [29] presented aggregated data from 131 flights where contact tracing was initiated following a suspected TB transmission. Test results were available for 758 contacts, including one TST converter and 11 other positive contacts with no risk factors for prior TB infection.

Only one study provided substantial evidence of TB transmission [16]: Six test-positive passengers with no other risk factors for test positivity, including four TST converters, were seated in the same aircraft section as the index case [16]. Four of these six test-positive passengers (including two TST converters) had been seated within two rows of the index patient, and two others reported having frequently visited friends during the flight who were seated very near the index patient. In addition, the index case had transmitted the disease to several household contacts before air travel. In the study by Miller and colleagues [21], all 34 test-positive contacts, including five converters, could be somewhat likely explained by BCG vaccination or prior exposure to TB in TB-endemic countries, but TST positivity was associated with sitting within one row's distance from the index case. No case of active TB following transmission on an aircraft has so far been reported.

One of the records identified included a smear-negative index case [3,4]. No evidence on transmission of the disease to other passengers or close contacts could be found. In six studies describing results of 10 contact investigations the index case was infected with an MDR or extensively drug-resistant (XDR) strain [3,4,14,16,18,20], however, only one flight provided evidence that transmission had possibly occurred [16]. An IGRA test was used in only three of the records. Thibeault et al. [24] found one IGRA-positive crew member among four that were tested, and Lynggaard et al. [19] reported one positive passenger among 16 who were tested with IGRA, and who was likely not to have contracted the infection during the flight. In one of the records [18] IGRAs were used but not reported separately from TSTs. Only one incident was found where the contact tracing had been started more than three months after the flight [18]. The type of aircraft was reported in seven of the 21 records [16,19,21-23,26,27] comprising 12 flights. On six of the aircraft a high-efficiency particulate air (HEPA) filter was used (data not shown).

### Estimation of the risk of transmission

Pooling the data from the records identified in the literature review where the contact tracing strategy included all passengers and crew [12,16,20-22,25,26], among a total of 1,287 aircraft contacts for whom a test result was available, 10 (0.8%) passengers were possibly infected during the flight (positives with no other risk factors for test positivity), seven (0.5%) of whom had a TST conversion. For incidents where only five rows surrounding the index case were traced [3,18,19,29], among a total of 905 aircraft contacts with test results, 12 (1.3%) passengers were possibly infected during the flight (positives with no other risk factors for test positivity), one (0.1%) of whom had a TST conversion. It should be noted that there were notable differences in proportions of contacts tested and diagnostic schemes, so these figures are only an estimate. Main reasons for unavailability of TB testing results were insufficient contact information, lost to follow up, residence in a foreign country and previous TB infection positivity. In addition, the infectiousness of the index patients varied across the records (see Table 1).

## Discussion

### Literature review

Based on currently available evidence, the risk of TB transmission during air travel is very low. In our study a rough estimate of 0.1–1.3% of aircraft contacts in long-haul flights (›eight hours) might have contracted the infection from a sputum-smear-positive index case. The risk of infection seems to be the highest among passengers seated within two rows of the index case.

In the studies performed before 2007, all passengers and crew were considered as contacts whereas in more recent studies only five rows in the proximity of the index case have been screened. The latter strategy has given a somewhat better yield of test-positive contacts (0.8% and 1.3%, respectively). Our estimates are likely biased due to the heterogeneity of the data. National authorities may have more success in tracing and testing contacts who are national residents. This will not necessarily alter the yield of the tested passengers but may alter the effectiveness of reaching all contacts. It is likely that the prevalence of test positivity before the flight is underestimated, and the transmission risk hence overestimated. In addition, in half of the studies it was not specifically mentioned whether household/close contacts were travelling with the index case and excluded from the results of the passenger investigation. If infected close contacts were included in the

**Table 2**

Comparison of criteria for risk assessment in European Centre for Disease Prevention and Control, World Health Organization, and Centers for Disease Control and Prevention guidelines on tuberculosis transmission on aircraft

| | ECDC/RAGIDA 2014 [35] | ECDC/RAGIDA 2009 [8] | WHO 2008 [2] | CDC 2012 [17] |
|---|---|---|---|---|
| Infectiousness | Same as in 2009 | Infectious pulmonary TB (smear-positive in spontaneous or induced sputum or bronchoalveolar lavage). | **Infectious TB**: all cases of respiratory (pulmonary or laryngeal) TB which are sputum smear-positive and culture-positive (if culture is available). **Potentially infectious TB:** all cases of respiratory (pulmonary or laryngeal) TB that are sputum smear negative and culture positive (susceptible, MDR-TB or XDR-TB). Additional information should be requested to conduct a risk assessment and determine whether a contact investigation should be considered. | Diagnosis of the index case was confirmed by sputum culture or nucleic acid amplification AND is:<br>(i) sputum smear-positive for acid-fast bacilli AND cavitation is present on a chest radiograph; OR<br>(ii) confirmed to have a multidrug-resistant isolate (regardless of the smear or chest radiograph. results). |
| M/XDR-TB | Same as 2009<br>Additionally, the infected contacts should be given advice on what actions to take if symptoms develop, such as informing the treating physician of the possibility of infection with a MDR strain. | No special considerations, the risk of infection of passengers with M/XDR-TB should be assessed using national guidelines. | Consequences of transmission of an M/XDR strain should be included in the risk assessment. | Stricter for MDR-TB (see previous row) |
| Pre-travel | Same as WHO 2008<br>Risk of infection of passengers with M/XDR-TB should be assessed using national guidelines. | Patients with confirmed infectious pulmonary TB should avoid air travel. If unavoidable, a specific travel protocol should be agreed upon. Risk of infection of passengers with M/XDR-TB should be assessed using national guidelines. | People with infectious or potentially infectious TB should not travel by commercial air transportation on a flight of any duration. | Not specifically mentioned |
| Evidence of transmission | Same as 2009<br>Additionally, if previous contact investigation results cannot be obtained despite considerable efforts, the tracing should be initiated only in exceptional circumstances. | Evidence of transmission to other contacts (refers to cases with evidence of transmission in household or other close contacts). | Documented transmission to close contacts is one of the criteria to consider in the risk assessment to decide whether a contact tracing is initiated if index case is classified as 'potentially infectious'. | Considered only in exceptional cases |
| Flight duration | Same as 2009 | ≥8 h (including ground delays) | Total flight duration ≥8 h (including ground delays after boarding, flight time and ground delays after landing) | ≥8 hours gate-to-gate (including boarding and deplaning time or delays on the tarmac) |
| Time passed since flight | Same as 2009<br>Additionally, relevant national authorities may consider longer time lags in specific cases. | Time to diagnosis less than three months | 3 months before notification | Index case was diagnosed within 3 months of the flight AND the flight occurred within 3 months of notification |
| Contacts to suggest screening to | Same as 2009. Addition: for wide aircrafts, only contacts seated within two seats may be included | Contacts seated in the same row, two rows ahead and two rows behind the index case | Contacts seated in the same row, two rows ahead and two rows behind the index case | Contacts seated in the same row, two rows ahead and two rows behind the index case |
| Special considerations for susceptible groups | Same as 2009 | If tracing initiated, special efforts should be made to trace particularly susceptible contacts, such as children/infants. | Timely medical examination, radiograph & follow-up regardless of the TST | Not specifically mentioned |

CDC: Centers for Disease Control and Prevention; ECDC: European Centre for Disease Prevention and Control; RAGIDA: Risk Assessment Guidelines for TB Transmitted on Aircraft; MDR-TB: multidrug-resistant tuberculosis; WHO: World Health Organization; XDR-TB: extensively-drug resistant tuberculosis.

flight-related contact tracing, a yield towards a higher risk value could have been obtained.

Additionally, the quality of all the evidence that we found varied from low to very low, due to the fact that it is generated only via observational studies with several types of challenges, such as lack of timely acquisition of passenger contact details and patient follow-up. Indeed, several studies highlighted the difficulty of obtaining complete passenger contact information [14,20,25]. Abubakar et al. found no association between notification delay from the date of flight to the notification to a public health authority within the range of 21 to 61 days and the availability of information from airlines (England and Wales 2007–2008) [28]. In Canada, availability of adequate passenger contact information from the airlines improved between 2006 and 2008 [30]. The approaches taken in the studies varied from descriptions of isolated incidents to routine data collection over several years. It can also be speculated that publication bias favours the studies where possible flight-related infections have been found and that published data represent a very small proportion of real exposure of travellers on aircraft since many countries may not carry out flight-related TB screening, or do not publish the results.

Marienau et al. estimated the in-flight TB transmission risk for contacts within two rows to vary between 1.1% and 24% using a large US dataset including 131 contact investigations with 758 passenger contacts tested [31]. However, a large proportion of the passengers considered to have contracted TB infection on an aircraft had other risk factors for TB infection or held a passport from a high-incidence country [29], so these risk rates might be overestimated. In a systematic review performed by Fox et al., the prevalence of latent TB infection among close contacts of TB patients (including other than smear-positive cases) in all types of settings was shown to be 28% in high-income environments and 45% in low- and middle-income environments, and 19% among casual contacts of TB cases in high-income settings [32]. This implies that the transmission risk of TB infection in aircraft is substantially lower than that in other settings. Although smear-negative patients have been shown to contribute to TB transmission rates in other settings [33], our literature search did not identify any in-flight transmission from smear-negative patients.

In two contact investigations the risk of acquiring TB infection during the flight was associated with sitting within two rows of the index case [16,21]. No new evidence concerning the number of rows/seats that should be screened was found to have been published after the launch of the first RAGIDA-TB guidelines in 2009. Most modern aircraft that re-circulate cabin air are equipped with HEPA filters although for small jets typically used on short-haul flights it is less common [34]. All the types of aircraft used on flights exceeding eight hours that were mentioned in the records included in the literature review were relatively recent models where HEPA filters were likely to have been employed. The cabin air flows downwards from the overhead outlets, limiting the potential exposure from a TB patient to the close environment [2].

It can also be noted that under a prospective literature search monitoring undertaken after the revision of the RAGIDA-TB and until 31 December 2015, using the same criteria, 23 new records were identified. None of these contained additional primary evidence on TB transmission on aircraft, and so no new records would have been included in an analysis extending to 31 December 2015.

## RAGIDA-TB update 2014 and comparison to World Health Organization and United States Centers for Disease Control and Prevention guidelines

An overview of modifications to the second edition of RAGIDA-TB is presented in Table 2. The RAGIDA-TB document with the complete risk assessment algorithm is available [35].

In regards to GRADE criteria, all decisions were based on evidence supplemented by expert opinion. The RAGIDA-TB 2013 expert group agreed that all modifications serve the best interest of the exposed passengers, balancing the chances of doing good with the chances of unnecessary testing while using resources wisely [11]. The expressed will of the exposed passengers, however, could not be assessed and is likely to vary substantially.

The evidence indicates that airline passengers exposed to a TB patient should not be considered as close contacts but rather as belonging to the second circle of contacts that is examined only if transmission to close contacts has occurred, following the principle of concentric circles of exposure [36]: A virtual ‘first circle’ of the most intensively exposed contacts is defined (usually reserved for prolonged contacts such as persons living and sleeping in the same room or under the same roof); one or more ‘outer’ circles with less exposed contacts are defined, with contacts to be investigated only if infected persons are found in the next inner circle. In view of the specificities of ventilation of modern passenger aircraft (air flow from roof to bottom in each segment, HEPA filters), which constantly removes air-borne particles and the limited amount of time spent even on long-haul flights, aeroplane passengers should not be considered to be in the innermost circle.

In support of this, the only study that provided considerable evidence on TB transmission occurring during air travel [16] reported that the index case had also transmitted the disease to closer contacts. However, in practice it can be difficult to obtain reliable information on the index case’s contact tracing results, and in many countries contact tracing is not carried out even for close contacts. Results of contact investigation may

only become available months after diagnosis and the discovery that the patient has been on a flight. In case this information cannot be obtained despite considerable efforts or will become available only later, contact tracing should be initiated only in exceptional circumstances.

In the scope of suspected in-flight transmission of TB, only cases with positive smear microscopy should be considered infectious. As there is no evidence of higher infectiousness of MDR-TB strains [37,38], the risk assessment for infection should be the same as for susceptible strains. However, as the potential consequences of an M/XDR-TB infection are more severe, the risk of transmission should be assessed using national guidelines. Individuals found to be potentially infected after exposure to an M/XDR-TB strain should be advised to inform the treating physician about the resistance status in case symptoms develop.

RAGIDA-TB recommends that contact investigations among passengers are initiated only if the index case is diagnosed within three months after the flight, due to the difficulties of assessing infectiousness at the time of the flight, interpreting test results to determine recent vs remote infection, and obtaining passenger travel and seating information [2,35]. The consideration of time passed between the flight and notification of the incident is left to the discretion of the relevant authorities; however, it should be kept in mind that the longer the notification delay, the poorer the results of the contact tracing will be. In addition, there is a possibility that the infection may have already progressed to active disease. The first edition of the 1998 WHO guidelines set the three-month limit on the grounds that information becomes more difficult to obtain after this time.

The recommended strategy for contact tracing in RAGIDA-TB follows the WHO guidelines [2], encompassing the passengers seated in the same row as the index case, and those two rows in front and two rows behind. Modelling studies have shown that the risk of contracting TB infection on an aircraft varies from low to moderate, and is the highest in the rows closest to the index case [39,40]. Based on the RAGIDA-TB 2013 expert group's opinion, the updated RAGIDA guidelines suggest, as a possibility to consider, limiting the contact investigation to fewer passengers (within two seats surrounding the index case instead of two rows) in the case of wide aircraft with many seats per row. If particularly susceptible individuals, such as infants and children, are identified among the contacts, special efforts should be given to trace them. Other particularly susceptible individuals among the passenger contacts, such as HIV-positive and diabetic persons, are usually impossible to identify. If this information is available, these contacts should be prioritised as is done with infants and children.

Table 2 compares the risk assessment guidelines for TB transmission on aircraft between RAGIDA-TB, WHO and CDC. The three sets of guidelines share many similarities in terms of criteria for initiating contact tracing, such as minimum flight duration and contact screening strategy. In addition, all three guideline documents stipulate that patients with untreated smear- or culture-positive pulmonary TB should not travel by air.

In contrast to the other two sets of guidelines, RAGIDA-TB recommends contact tracing only if there is already evidence of transmission from the smear-positive index case to close contacts outside of the aircraft setting, as discussed above. While WHO recommends assessing the risk of transmission to passengers from infectious (sputum and culture positive) as well as potentially infectious (culture-positive but smear-negative) patients, RAGIDA-TB only considers index cases that are positive by microscopy in spontaneously produced or induced sputum or bronchoalveolar lavage. Further, the CDC guidelines recommend that for index cases with MDR-TB, contact tracing should be performed even for smear-negative patients.

The CDC guidelines were revised in 2011 [17]. According to the updated criteria, contact investigations should be initiated if the index case is smear and cavitation positive, whereas in the previous 2008 CDC guidelines only smear-positivity was required. In addition, the maximum time elapsed between flight and notification has been shortened from six months to three months. The revision therefore results in a smaller number of contact investigations. The comparative public health risk of the effects of the revision has been analysed against benefits of cost savings, concluding that the more exclusive protocol imposes minimal risks to public health while requiring only half of the costs and is more beneficial from both epidemiological and economic perspectives [31,41].

According to the UK National Institute for Health and Care Excellence (NICE) guidelines contact tracing of passengers should not be undertaken routinely [42]: instead, the passengers seated close to the index case should be provided with information on the risk of TB and what actions to take if symptoms develop. To our knowledge, the guidelines issued by Public Health Agency of Canada are the most stringent; according to these, contact investigation is initiated even in the case of smear-negative index patients when there are no data available to indicate that transmission did not occur in non-flight contacts [43]. In addition, the contact investigation should be started regardless of the time passed between the flight and the notification of the incident, and for cases of MDR, XDR and laryngeal TB regardless of duration of the flight if there is insufficient data to exclude transmission to non-flight contacts.

## Conclusions

This systematic literature review compiled the most up-to-date evidence base on transmission of TB during air

travel. We identified observational studies providing only low-quality evidence, but it can still be concluded that the risk of TB transmission on aircraft seems to be very low. Despite the lack of good quality data, the RAGIDA-TB 2013 expert group concluded that this is not a research gap that should be prioritised and TB research resources are better directed elsewhere.

The RAGIDA-TB update resulted in clear and evidence-focused guidelines which will help to use resources in an effective way [35]. These guidelines provide a clear framework for risk assessment but leave room for flexibility in unusual cases. There is notable variation and opportunities remain for improvement via harmonisation between different national and supranational TB guidelines for risk assessment of transmission on aircraft.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## Conflict of interest

None declared.

## Authors' contributions

Study concept and design: I. Abubakar, L. Payne Hallström, S.M. Kotila. Analysis and/or interpretation of the data: all authors. S.M. Kotila had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. S.M. Kotila and L. Payne Hallström reviewed the records found in the literature search to select the relevant records. All authors had access to the extracted data. Drafting of the manuscript: S.M. Kotila. Revision of the manuscript for important intellectual content: I. Abubakar, L. Payne Hallström, P. Helbling, N. Jansen.

## References


1. Outlook for Air Transport to the Year 2015. Montreal: International Civil Aviation Organization (ICAO); 2004. Available from: aviation.mid.gov.kz/sites/default/files/pages/304_prognoz_razvitiya_vozdushnogo_transporta_do_2015_goda_en_1.pdf
2. Tuberculosis and air travel: guidelines for prevention and control. Geneva: World Health Organization; 2008. Available from: http://www.who.int/tb/publications/2008/WHO_HTM_TB_2008.399_eng.pdf
3. Buff AMSD, Scholten D, Rivest P, Hannah H, Marshall J, Wiersma P, et al. Multinational investigation of a traveler with suspected extensively drug-resistant tuberculosis—2007. 12th Annual Conference of the International Union Against Tuberculosis and Lung Disease-North American Region; 28 February–1 March 2008; San Diego, CA, USA. Vancouver, BC, Canada: British Columbia Lung Association; 2008. p. 11.
4. Tuberculosis Team (ECDC). Preparedness and Response Unit (ECDC). Airline traveller with extensively drug-resistant tuberculosis: low risk for passengers.Euro Surveill. 2007;12(22).
5. Gibbs N. Plague on a plane.Time. 2007;169(24):19.PMID: 18655534
6. Parmet WE. Legal power and legal rights--isolation and quarantine in the case of drug-resistant tuberculosis.N Engl J Med. 2007;357(5):433-5. DOI: 10.1056/NEJMp078133 PMID: 17671251
7. Tanne JH. Tuberculosis case exposes flaws in international public health systems.BMJ. 2007;334(7605):1187. DOI: 10.1136/bmj.39237.452269.DB PMID: 17556459
8. European Centre for Disease Prevention and Control (ECDC). Risk assessment guidelines for infectious diseases transmitted on aircraft. Stockholm: ECDC, 2009. Available from: http://ecdc.europa.eu/en/publications/publications/1012_gui_ragida_2.pdf
9. Abubakar I. Tuberculosis and air travel: a systematic review and analysis of policy.Lancet Infect Dis. 2010;10(3):176-83. DOI: 10.1016/S1473-3099(10)70028-1 PMID: 20185096
10. Leitmeyer K, Payne Hallstrom L, Danielsson N, Carrillo Santisteve P. Risk assessment guidance for infectious diseases on aircraft (RAGIDA) – are we up to date? 2014 European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE). Stockholm, Sweden; 2014.
11. GRADE Working Group,Guyatt GH, Oxman AD, Vist GE, Kunz R, Falck-Ytter Y, Alonso-Coello P, et al. . GRADE: an emerging consensus on rating quality of evidence and strength of recommendations.BMJ. 2008;336(7650):924-6. DOI: 10.1136/bmj.39489.470347.AD PMID: 18436948
12. Beller M. Tuberculosis and Air Travel.State of Alaska Epidemiology Bulletin. 1996; Bulletin No. 27. Available from: http://www.epi.alaska.gov/bulletins/docs/b1996_27.htm.
13. From the Centers for Disease Control and Prevention. Exposure of passengers and flight crew to Mycobacterium tuberculosis on commercial aircraft, 1992-1995. JAMA. 1995;273(12):911-2. DOI: 10.1001/jama.1995.03520360023016 PMID: 7884939
14. Chemardin J, Paty MC, Renard-Dubois S, Veziris N, Antoine D. Contact tracing of passengers exposed to an extensively drug-resistant tuberculosis case during an air flight from Beirut to Paris, October 2006. Euro Surveill. 2007;12:E071206 2.
15. Driver CR, Valway SE, Morgan WM, Onorato IM, Castro KG. Transmission of Mycobacterium tuberculosis associated with air travel.JAMA. 1994;272(13):1031-5. DOI: 10.1001/jama.1994.03520130069035 PMID: 8089885
16. Kenyon TA, Valway SE, Ihle WW, Onorato IM, Castro KG. Transmission of multidrug-resistant Mycobacterium tuberculosis during a long airplane flight.N Engl J Med. 1996;334(15):933-8. DOI: 10.1056/NEJM199604113341501 PMID: 8596593
17. Centers for Disease Control and Prevention (CDC),Kim C, Buckley K, Marienau KJ, Jackson WL, Escobedo M, Bell TR, et al. . Public health interventions involving travelers with tuberculosis--U.S. ports of entry, 2007-2012.MMWR Morb Mortal Wkly Rep. 2012;61(30):570-3.PMID: 22854625
18. Kornylo-Duong K, Kim C, Cramer EH, Buff AM, Rodriguez-Howell D, Doyle J, et al. Three air travel-related contact investigations associated with infectious tuberculosis, 2007-2008. Travel Med Infect Dis. 2010;8(2):120-8. DOI: 10.1016/j.tmaid.2009.08.001 PMID: 20478520
19. Lynggaard CD, Eriksen NM, Andersen PH, David KP. [Tracing exposed flight passengers after discovery of pulmonary tuberculosis]. Ugeskr Laeger. 2011;173(12):899-900.PMID: 21419062
20. McFarland JW, Hickman C, Osterholm M, MacDonald KL. Exposure to Mycobacterium tuberculosis during air travel.Lancet. 1993;342(8863):112-3. DOI: 10.1016/0140-6736(93)91311-9 PMID: 8100876
21. Miller MA, Valway S, Onorato IM. Tuberculosis risk after exposure on airplanes.Tuber Lung Dis. 1996;77(5):414-9. DOI: 10.1016/S0962-8479(96)90113-6 PMID: 8959144
22. Moore M, Fleming KS, Sands L. A passenger with pulmonary/laryngeal tuberculosis: no evidence of transmission on two short flights.Aviat Space Environ Med. 1996;67(11):1097-100. PMID: 8908350
23. Parmet AJ. Tuberculosis on the flight deck.Aviat Space Environ Med. 1999;70(8):817-8.PMID: 10447057
24. Thibeault C, Tanguay F, Lacroix C, Menzies R, Rivest P. A case of active tuberculosis in a cabin crew: the results of contact tracing.Aviat Space Environ Med. 2012;83(1):61-3. DOI: 10.3357/ASEM.3135.2012 PMID: 22272519
25. Vassiloyanakopoulos A, Spala G, Mavrou E, Hadjichristodoulou C. A case of tuberculosis on a long distance flight: the difficulties of the investigation.Euro Surveill. 1999;4(9):96-7. PMID: 12631891

26. Wang PD. Two-step tuberculin testing of passengers and crew on a commercial airplane.Am J Infect Control. 2000;28(3):233-8. DOI: 10.1067/mic.2000.103555 PMID: 10840343
27. Whitlock G, Calder L, Perry H. A case of infectious tuberculosis on two long-haul aircraft flights: contact investigation.N Z Med J. 2001;114(1137):353-5.PMID: 11587303
28. Abubakar I, Welfare R, Moore J, Watson JM. Surveillance of air-travel-related tuberculosis incidents, England and Wales: 2007-2008.Euro Surveill. 2008;13(23).PMID: 18761951
29. Marienau KJ, Burgess GW, Cramer E, Averhoff FM, Buff AM, Russell M, et al. Tuberculosis investigations associated with air travel: U. S. Centers for Disease Control and Prevention, January 2007-June 2008. Travel Med Infect Dis. 2010;8(2):104-12. DOI: 10.1016/j.tmaid.2010.02.003 PMID: 20478518
30. Scholten D, Saunders A, Dawson K, Wong T, Ellis E. Air travel by individuals with active tuberculosis: reporting patterns and epidemiologic characteristics, Canada 2006-2008.Travel Med Infect Dis. 2010;8(2):113-9. DOI: 10.1016/j.tmaid.2010.02.002 PMID: 20478519
31. Marienau KJ, Cramer EH, Coleman MS, Marano N, Cetron MS. Flight related tuberculosis contact investigations in the United States: comparative risk and economic analysis of alternate protocols.Travel Med Infect Dis. 2014;12(1):54-62. DOI: 10.1016/j.tmaid.2013.09.007 PMID: 24206902
32. Fox GJ, Barry SE, Britton WJ, Marks GB. Contact investigation for tuberculosis: a systematic review and meta-analysis.Eur Respir J. 2013;41(1):140-56. DOI: 10.1183/09031936.00070812 PMID: 22936710
33. Tostmann A, Kik SV, Kalisvaart NA, Sebek MM, Verver S, Boeree MJ, et al. Tuberculosis transmission by patients with smear-negative pulmonary tuberculosis in a large cohort in the Netherlands. Clin Infect Dis. 2008;47(9):1135-42. DOI: 10.1086/591974 PMID: 18823268
34. United States Government Accountability Office (GAO). Aviation Safety. More Research Needed on the Effects of Air Quality on Airliner Cabin Occupants. Report to the Ranking Democratic Member, Subcommittee on Aviation, Committee on Transportation and Infrastructure, House of Representatives. GAO-04-54. Washington, DC: GAO; 2004. Available from: http://www.gao.gov/products/GAO-04-54
35. European Centre for Disease Prevention and Control (ECDC). Risk assessment guidelines for infectious diseases transmitted on aircraft (RAGIDA) – Tuberculosis. Stockholm: ECDC; 2014. Available from: http://ecdc.europa.eu/en/publications/Publications/tuberculosis-risk-assessment-guidelines-aircraft-May-2014.pdf
36. Erkens CG, Kamphorst M, Abubakar I, Bothamley GH, Chemtob D, Haas W, et al. Tuberculosis contact investigation in low prevalence countries: a European consensus. Eur Respir J. 2010;36(4):925-49. DOI: 10.1183/09031936.00201609 PMID: 20889463
37. Cohen T, Murray M. Modeling epidemics of multidrug-resistant M. tuberculosis of heterogeneous fitness.Nat Med. 2004;10(10):1117-21. DOI: 10.1038/nm1110 PMID: 15378056
38. Borrell S, Gagneux S. Infectiousness, reproductive fitness and evolution of drug-resistant Mycobacterium tuberculosis.Int J Tuberc Lung Dis. 2009;13(12):1456-66.PMID: 19919762
39. Jones RM, Masago Y, Bartrand T, Haas CN, Nicas M, Rose JB. Characterizing the risk of infection from Mycobacterium tuberculosis in commercial passenger aircraft using quantitative microbial risk assessment.Risk Anal. 2009;29(3):355-65. DOI: 10.1111/j.1539-6924.2008.01161.x PMID: 19076326
40. Ko G, Thompson KM, Nardell EA. Estimation of tuberculosis risk on a commercial airliner.Risk Anal. 2004;24(2):379-88. DOI: 10.1111/j.0272-4332.2004.00439.x PMID: 15078308
41. Coleman MS, Marienau KJ, Marano N, Marks SM, Cetron MS. Economics of United States tuberculosis airline contact investigation policies: a return on investment analysis. Travel Med Infect Dis. 2014;12(1):63-71. DOI: 10.1016/j.tmaid.2013.10.016 PMID: 24262643
42. National Institute for Health and Care Excellence (NICE). Tuberculosis: clinical diagnosis and management of tuberculosis, and measures for its prevention and control. NICE guidelines [CG117]. London: NICE; 2011. Available from: http://www.nice.org.uk/guidance/cg117/chapter/guidance
43. Public Health Agency of Canada (PHAC). Canadian Tuberculosis and Air Travel Guidelines. Version 2.0, 2009. Ottawa: PHAC; 2009. Available from: http://www.phac-aspc.gc.ca/tbpc-latb/pdf/guideform_tbaircraft09-eng.pdf

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