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Zika virus infections in three travellers returning from South America and the Caribbean respectively, to Montpellier, France, December 2015 to January 2016

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We report three unrelated cases of Zika virus infection in patients returning from Martinique, Brazil and Colombia respectively, to Montpellier, France. They developed symptoms compatible with a mosquito-borne disease, and serological and molecular investigations indicated a recent Zika virus infection. Considering the recent warning for the likely teratogenicity of Zika virus and the presence of competent mosquito vectors in southern France, these cases highlight the need for awareness of physicians and laboratories in Europe.

Since early 2015 there has been a rapid spread of Zika virus infections in South America with a subsequent threat for importation of that emerging disease in other regions of the world. Here we describe three cases in travellers returning to France from affected areas.

Description of cases

Case 1

On 24 December 2015, a woman in her sixties presented at the Department of Infectious and Tropical Diseases at the University Hospital of Montpellier, France. Three days earlier she had developed sudden fever associated with myalgia, maculopapular rash located on face, trunk and limbs, and conjunctivitis. Symptoms onset occurred two days after having returned from a three-week vacation on Martinique Island (French West Indies). Blood cell count, liver enzymes and renal function were normal. Fever and rash resolved on day 3, but fatigue and muscular symptoms lasted for seven days. Zika virus (ZIKV) real-time polymerase chain reaction (RT-PCR) was negative in blood on day 5 after symptom onset; urine samples were not collected for testing. ZIKV IgM antibodies were detected on 24 December

(day 5 after symptom onset) with an increasing level in subsequent samples, whereas the rise of ZIKV IgG antibodies was noticed three weeks later.

Case 2

On 13 January 2016, a man in his twenties was examined in the same department. He had experienced gradual onset of fever, myalgia, diarrhoea, arthralgia and cutaneous rash on trunk and limbs, starting on 5 January, one day after his return from a one-week stay in Rio de Janeiro, Brazil. Upon examination in hospital, fever and cutaneous rash had disappeared, but arthralgia persisted, in association with asthenia, non-productive cough and conjunctivitis. On the day of admission (13 January), laboratory tests showed normal blood cell count and normal renal function, while transaminases were slightly increased. RT-PCR for ZIKV was negative in blood and urine samples. ZIKV IgG and IgM antibodies were detected in serum concomitantly with DENV antibodies; however, the specificity of these anti-ZIKV antibodies was confirmed by a neutralisation assay. Three of the patient's relatives living in Brazil were concurrently diagnosed with symptomatic ZIKV infection.

Case 3

A man in his fifties progressively developed myalgia in lower limbs, pruriginous rash and fever, two days after returning from a three-week vacation in Columbia. He was examined in the same hospital department on the third day (13 January), and showed intense fatigue, extensive maculopapular eruption on the face, trunk and both upper and lower limbs, ulcerative pharyngitis, and conjunctivitis. Results of the neurological examination were normal. Blood cell count showed mild leucopenia (3,800 cells/ μ L; norm: $>4,000$ cells/ μ L),

TABLE

Temporal and virological data related to three imported cases of Zika virus infection, Montpellier, France, December 2015 to January 2016

Cases		Case 1	Case 2	Case 3
Temporal information				
Returning country		Martinique	Brazil	Colombia
Duration of stay		3 weeks	1 week	4 weeks
Date of return		18 Dec 2015	4 Jan 2016	10 Jan 2016
Symptoms onset		20 Dec 2015	5 Jan 2016	11 Jan 2016
Viral investigation				
First sample date		24 Dec 2015 (D5)	13 Jan 2016 (D9)	13 Jan 2016 (D3)
Dengue virus	RT-PCR ^a plasma	Negative	Negative	Negative
	RT-PCR urine	NS	Negative	Negative
	IgM ^b (OD ^c 1/200)	Negative (0.096)	Positive (0.241)	Positive (0.106)
	IgG ^d (OD 1/500)	Negative (0.061)	Positive (1.139)	Positive (0.209)
Chikungunya virus	RT-PCR ^a plasma	Negative	Negative	Negative
	RT-PCR urine	NS	Negative	Negative
	IgM ^b (OD 1/200)	Negative (0.077)	Negative (0.089)	Negative (0.064)
	IgG ^d (OD 1/500)	Negative (0.048)	Negative (0.047)	Negative (0.052)
Zika virus	RT-PCR ^a plasma	Negative	Negative	Positive (Ct=37.0)
	RT-PCR urine	NS	Negative	Positive (Ct=33.2)
	IgM ^b (OD 1/200)	Positive (0.264)	Positive (0.501)	Negative (0.104)
	IgG ^d (OD 1/500)	Negative (0.047)	Positive (0.301)	Negative (0.061)
Second sample date		4 Jan 2016 (D14)	18 Jan 2016 (D14)	18 Jan 2016 (D8)
Dengue virus	IgM (OD 1/200)	Negative (0.095)	Negative (0.191)	Positive (0.364)
	IgG (OD 1/500)	Negative (0.049)	Positive (0.899)	Positive (0.823)
Zika virus	RT-PCR plasma	ND	Negative	Negative
	RT-PCR urine	NS	Negative	Positive (Ct=33.9)
	RT-PCR saliva	NS	Negative	Positive (Ct=30.3)
	IgM (OD 1/200)	Positive (0.895)	Positive (0.446)	Positive (0.433)
	IgG (OD 1/500)	Negative (0.065)	Positive (0.406)	Positive (0.368)
	Zika virus neutralising antibodies ^e	1/320 (<1/40)	1/640 (1/160)	1/40 (<1/40)
Third sample date		14 Jan 2016 (D21)	NS	NS
Zika virus	IgM (OD 1/200)	Positive (0.313)	NS	NS
	IgG (OD 1/500)	Positive (0.155)	NS	NS
	Zika virus neutralising antibodies ^e	1/640 (<1/40)	NS	NS

Ct: Cycles threshold; D: days from symptom onset; ND: not determined; NS: not sampled; OD: optical density; RT-PCR: real-time polymerase chain reaction.

^a RT-PCRs were performed with the RealStar dengue RT-PCR kit 2.0, the RealStar chikungunya RT-PCR kit 1.0 and the RealStar Zika virus RT-PCR kit 1.0 (Altona Diagnostic, Germany).

^b Flaviviruses IgM and chikungunya virus IgM detections were performed with in house IgM antibody-capture ELISA (MAC-ELISA) assays.

^c At 1/200 or 1/500 working dilutions.

^d Flaviviruses IgG and chikungunya virus IgG detections were performed with in house indirect ELISA assays.

^e Zika virus neutralising antibodies (result of West Nile virus neutralisation antibodies assay performed as control) with the titre of serum neutralising 90%.

with normal liver enzymes and renal function. RT-PCR for ZIKV was positive in blood, urine and saliva samples. ZIKV seroconversion was detected in the second sample (day 8 after symptom onset) with observation of cross-reactivity with flaviviruses including dengue. Interestingly, two relatives who travelled with him were subsequently tested, and the results were negative for ZIKV.

Symptoms disappeared completely within one week in all patients. Temporal and viral investigation data are summarised in the Table.

Background

Zika virus is a mosquito-borne flavivirus related to dengue virus (DENV), yellow fever virus (YFV) and West Nile virus (WNV). A large outbreak of ZIKV infections involving the ZIKV Asian lineage is ongoing in Brazil since April 2015 [1] with up to 18 countries affected as

at 23 December 2015 [2]. By the first week of December 2015, nine additional South American countries and Cape Verde islands had reported locally acquired cases [3]. Furthermore, a link between ZIKV infection and neurological disorders or congenital malformations has been suspected in Brazil, and an epidemiological alert from the Pan American Health Organization (PAHO) has been issued [4]. ZIKV which is transmitted by *Aedes aegypti* has been isolated from several *Aedes* mosquito species [5] and transmission by *Ae. albopictus* has been documented in Gabon [6], leading to the threat of a worldwide spread. In the last week of December, the French Ministry of Health issued a warning about the detection of autochthonous cases of ZIKV infections in French Departments of America, French Guyana and Martinique Island, confirming the spread of ZIKV in the Caribbean [7]. Given that South American and Caribbean countries are highly touristic regions and that European overseas districts in that area have close connections with their related European mainland countries, there is a risk for imported cases to occur in Europe.

Discussion and conclusions

No autochthonous case of ZIKV infection and a limited number of cases related to the South American outbreak have been reported so far in Europe. The first one was observed in Italy, at the beginning of the Brazilian outbreak, in a traveller returning from Brazil [8] and, more recently, in November 2015, in a traveller returning to the Netherlands from Surinam [3]. Interestingly, similarly to Case 1 returning from Martinique, these imported cases were concomitantly detected close to the first reported locally-acquired cases. Since most ZIKV infections are asymptomatic or mild, this suggests that, at the time of the first autochthonous cases, the overall burden of ZIKV infection has been underestimated.

Since its first introduction in 2004, the mosquito vector *Ae. albopictus* has been well established in southern France. Autochthonous transmissions of chikungunya virus (CHIKV) or DENV previously occurred in Europe [9,10], such as in Montpellier, with an outbreak of 12 locally acquired CHIKV infections in October 2014 [11] or in Nîmes, a nearby town, with an outbreak of six autochthonous DENV infection cases in 2015 [12]. Thus, prerequisites for ZIKV autochthonous transmission are likely met in southern France. However, despite the fact that *Ae. albopictus* is an *in vitro* competent vector for the ZIKV African lineage [13] and was identified as an efficient vector for this lineage in Gabon [6], its vectorial capacity for the ZIKV Asian lineage remains to be clarified. Furthermore, in the cases reported here, the risk of local transmission can be ruled out, considering the vector inactivity during winter time.

However, this description of imported cases, including one case from a French Overseas Department, should reinforce the preparedness plan for arboviral outbreaks which is implemented each year since

2006, during the *Ae. albopictus* activity period (May to November), in all *Ae. albopictus*-colonised areas in France [11]. This means that the network of laboratories that currently propose CHIKV and DENV diagnosis should add ZIKV diagnosis to their panel, with regular reports to regional surveillance boards, and that practitioners' awareness of clinically-suspected cases must be raised; moreover, they should be required to report to regional health authorities. However, as illustrated here, the laboratory diagnosis of ZIKV infection might be challenging due to the transient viraemia, the antibody rise that might be delayed, and the IgG flavivirus cross-reactivity that may interfere in serological testing. This will be a concern for the surveillance of pregnant women [14] as well as for blood safety policy [15].

Conflict of interest

None declared.

Authors' contributions

Managed the patients: ATM, AM, VLM; performed laboratory investigations: MM, OF, ILG, VF; wrote the manuscript: ATM, MS, VLM, VF.

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Prevalence of *mcr-1* in commensal *Escherichia coli* from French livestock, 2007 to 2014

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Colistin resistance was investigated in 1,696 isolates collected from 2007 to 2014 within the frame of the French livestock antimicrobial resistance surveillance programme. The *mcr-1* gene was detected in all commensal *Escherichia coli* isolates with a minimum inhibitory concentration to colistin above the 2 mg/L cut-off value ($n=23$). In poultry, *mcr-1* prevalence was 5.9% in turkeys and 1.8% in broilers in 2014. In pigs, investigated in 2013, this prevalence did not exceed 0.5%. These findings support that *mcr-1* has spread in French livestock.

We report *mcr-1* prevalence data in commensal *Escherichia coli* isolated from French livestock from 2007 to 2014.

Laboratory investigation

According to the European Union surveillance programme on antimicrobial resistance in zoonotic and commensal bacteria (directive 2003/99/EC) [1], a random sample of faecal (until 2013) or caecal (since 2014) content from the same epidemiological unit (defined as in [2]) of broilers, pigs and turkeys was taken at slaughter houses all over the country, in order to be representative of national productions. The sampling was proportional to the slaughter houses' annual throughputs and was spread over the year. The number of samples collected per animal species and year was calculated to be able to recover at least 170 *E. coli* isolates for each combination of bacterial species and animal production. Isolates were streaked on MacConkey medium, identified and tested for antimicrobial susceptibility by the broth microdilution method (Trek diagnostic systems) using a panel of 14 antimicrobial substances. The minimum inhibitory concentrations (MIC) obtained were compared with the epidemiological cut-off values of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [3]. The DNA of strains with a colistin MIC over 2 mg/L was extracted and the presence of *mcr-1* sought by polymerase chain reaction (PCR) [4].

Colistin resistance and presence of the *mcr-1* gene in isolates

Most (1,427/1,450; 98%) commensal *E. coli* strains isolated and tested from French livestock between 2007 and 2014 were susceptible to colistin (Table).

During the study period however, a total of 23 isolates were resistant to colistin at concentrations above the cut-off value of 2 mg/L, with MICs ranging from 4 to 16 mg/L. Interestingly, each individual *E. coli* isolate from French livestock with a MIC to colistin greater than the cut-off harboured the *mcr-1* gene. From 2011 to 2013, two strains resistant to colistin were isolated from healthy pigs. The prevalence of colistin resistance in broilers was 1.8% in 2014. In turkey production, monitoring commensal *E. coli* became mandatory at European level in 2014 and the prevalence of resistance to colistin was 5.9% that year. Co-resistance patterns were diverse, ranging from one to eight associated mechanism of resistance (data not shown). Nevertheless, in four of the 14 *mcr-1* positive turkey isolates, colistin resistance coincided with simultaneous resistance to ampicillin, quinolones, sulfamethoxazole, tetracycline and trimethoprim (data not shown). One single strain derived from turkeys was also resistant to cefotaxime and carrying the *bla_{CMY-2}* gene. Plasmid profiling in order to assess the transferability of these *mcr-1* genes from food producing animals to other hosts such as humans is under progress.

Discussion

For decades, colistin has been widely used in veterinary medicine against infections caused by *Enterobacteriaceae* in food-producing animals in Europe [5]. To offset limited data on colistin resistance in European livestock, this antibiotic was added in 2014 to the antimicrobial substances required to be tested under antimicrobial resistance programmes conducted by European Member States (decision 2013/652/EU [2]).

TABLE

Colistin resistant and *mcr-1* positive commensal *Escherichia coli* strains from French livestock, France, 2007–2014

Year	Animals	<i>E. coli</i> strains tested for MIC N	<i>E. coli</i> strains resistant to colistin N	Proportion of <i>mcr-1</i> positive (n) among colistin-resistant <i>E. coli</i> strains (N) n/N	Prevalence of <i>mcr-1</i> positive <i>E. coli</i> strains % (95%CI)
2014	Turkeys	239	14	14/14	5.9 (2.9–8.8)
	Broilers	227	4	4/4	1.8 (0.1–3.5)
2013	Pigs	196	1	1/1	0.5 (0.0–1.5)
	Broiler	193	3	3/3	1.6 (0.0–3.3)
2012	Pigs	194	0	N.a.	N.a.
	Broiler	201	0	N.a.	N.a.
2011	Pigs	200	1	1/1	0.5 (0.0–1.5)
2007	Turkeys	ND ^a	ND ^a	0/246 ^a	0 (0.0–1.2)
Total	All	1,450	23	N.a.^a	N.a.^a

CI: confidence interval; MIC: minimum inhibitory concentration; N.a.: not applicable; ND: not determined.

^aAs susceptibility to colistin was not tested in 2007, each isolate obtained in that year was tested for the presence of *mcr-1*.

In spite of this, prior to 2015, the mechanism of resistance to colistin was only known to involve chromosomal mutations, and so its spread was expected to be limited to vertical transmission [6]. In 2015 however, the first plasmid-mediated colistin resistance involving the *mcr-1* gene was discovered in China by Liu et al. [4]. Since, other reports detail retrospective detection of this gene in *E. coli* from animal origin. In Germany, the gene was found in three of the 129 whole-genome sequences of *E. coli* isolated from livestock since 2009 [7]. The *mcr-1* positive strains originated from swine and were sampled in 2010 and 2011. The *mcr-1* gene was also detected in five *E. coli* isolates from chicken meat of European origin imported in Denmark in 2012, 2013 and 2014 [8]. In Belgium, 13 of 105 colistin-resistant *E. coli* isolates collected in 2011 and 2012 from piglets and bovine calves with diarrhoea were positive for *mcr-1* [9]. Also, in France, extended-spectrum beta-lactamase (ESBL)-positive *E. coli* isolated from diarrhoeic bovine calves as early as 2005 were confirmed to be *mcr-1* positive [10] as well as four *Salmonella* isolates from 2012 to 2013 collected within the French agricultural food sector [11]. A number of these findings implicated pathogenic strains, isolated in the context of event-based surveillance networks or programmes.

Prompted by these reports of *mcr-1*-mediated colistin resistance, we investigated the prevalence of *mcr-1* in non-pathogenic *E. coli* isolated through the official European surveillance programme on antimicrobial resistance in French livestock. This programme is designed to be comparable between Member States but its power to detect emergent resistance is likely to be limited. In fact, after three years of continuous monitoring, starting from an initial theoretical point of 0.1% of resistant isolates, this programme cannot detect any changes if the overall increase is lower than 2% per year [2]. The fact that *mcr-1* emergence is detected through this surveillance programme supports the idea

of a rapid spread of plasmid-mediated colistin resistance in French livestock.

The presence of co-resistances in strains harbouring the *mcr-1* gene could have contributed to select and enhance the rapid dissemination of the plasmid-mediated resistance to colistin jointly with antibiotic pressure by other antimicrobial use in food producing animals.

The dissemination of *mcr-1* in French livestock, either in a pathogenic or healthy context, raises the question of colistin use in animals. Colistin use should be now revisited in a double perspective: first, in a veterinary medicine perspective, that might suddenly start to face treatment failures in animal digestive disorders such as colibacillosis or salmonellosis; and second, in a human medicine perspective, in order to maintain the efficacy of a last-resort therapeutic option to counteract multidrug-resistant bacterial infections [5].

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Conflict of interest

None declared.

Authors' contributions

APG designed the study, analysed and interpreted data, drafted and coordinated the manuscript elaboration, MB analysed the data and contributed to the manuscript, PH, KD, PL, CP produced phenotypic and molecular data, CS contributed to the manuscript, PS contributed to the manuscript and given scientific advice.

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Inverse trends of *Campylobacter* and *Salmonella* in Swiss surveillance data, 1988–2013

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Clinical isolates of *Campylobacter* spp. and *Salmonella* spp. are notifiable in Switzerland. In 1995, *Campylobacter* replaced *Salmonella* as the most frequently reported food-borne pathogen. We analysed notification data (1988–2013) for these two bacterial, gastrointestinal pathogens of public health importance in Switzerland. Notification rates were calculated using data for the average resident population. Between 1988 and 2013, notified campylobacteriosis cases doubled from 3,127 to 7,499, while *Salmonella* case notifications decreased, from 4,291 to 1,267. Case notifications for both pathogens peaked during summer months. *Campylobacter* infections showed a distinct winter peak, particularly in the 2011/12, 2012/13 and 2013/14 winter seasons. *Campylobacter* case notifications showed more frequent infection in males than females in all but 20–24 year-olds. Among reported cases, patients' average age increased for campylobacteriosis but not for salmonellosis. The inverse trends observed in case notifications for the two pathogens indicate an increase in campylobacteriosis cases. It appears unlikely that changes in patients' health-seeking or physicians' testing behaviour would affect *Campylobacter* and *Salmonella* case notifications differently. The implementation of legal microbiological criteria for foodstuff was likely an effective means of controlling human salmonellosis. Such criteria should be decreed for *Campylobacter*, creating incentives for producers to lower *Campylobacter* prevalence in poultry.

Introduction

Campylobacter spp. and *Salmonella* spp. are the most frequently reported zoonotic infections in Switzerland. The Federal Office of Public Health (FOPH) monitors communicable diseases in Switzerland. The National Notification System for Infectious Diseases (NNSID) is an integral part of ensuring compliance with this obligation and was implemented nationwide, in a standardised way, in 1987. The regulation on communicable

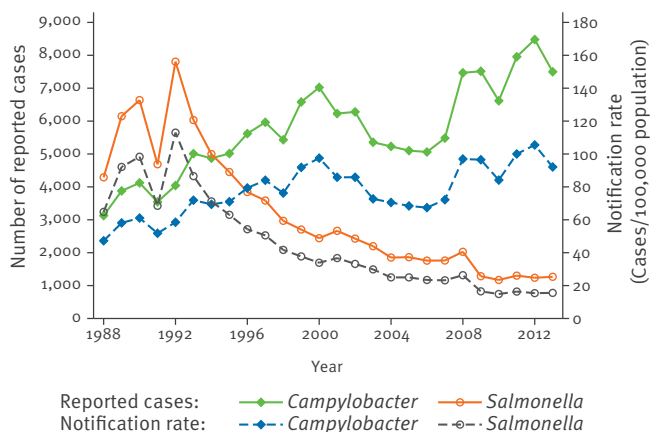
disease notifications determines which diseases have to be reported, by whom and in what timeframe [1]. Among food-borne pathogens, *Campylobacter* spp., *Salmonella* spp., *Listeria* spp., enterohaemorrhagic *Escherichia coli*, *Shigella* spp., and hepatitis A virus are notifiable. Laboratories must report isolates of *Campylobacter* and *Salmonella* within one week of discovery. For patients with suspected bacterial diarrhoea, basic stool culture including *Campylobacter* spp., *Salmonella* spp. and *Shigella* spp. is the routine method of laboratory diagnosis [2].

In humans, campylobacteriosis is most frequently caused by *Campylobacter jejuni* and *C. coli* [3]. Signs and symptoms include watery or bloody diarrhoea, fever, abdominal cramps, vomiting and malaise and usually occur after an incubation period of 2–5 days [4]. The disease usually resolves without antibiotic treatment within one week. A recent study on determinants of the disease in Switzerland showed that laboratory-confirmed campylobacteriosis can lead to severe illness in patients [5]. Complications such as Guillain-Barré syndrome can follow *Campylobacter* infections, although this is rare [4,6]. Fatal cases are possible, but the reported case fatality rate of 0.1% is small and four times lower than the fatality rate for salmonellosis [7].

There are more than 2,600 serovars of *Salmonella*, of which *S. enterica* subspecies *enterica* serovars Enteritidis (*S. Enteritidis*) and Typhimurium (*S. Typhimurium*) are the most frequently reported [8]. Signs and symptoms of salmonellosis are similar to those of campylobacteriosis but the incubation period is shorter at 6–72 hours (usually 12–36 hours) [9]. In a group of volunteers, the minimal infectious dose was found to be at least 200 times higher for *Salmonella* than for *Campylobacter* (10^5 – 10^9 vs 500 organisms) [10]. However, *Salmonella* outbreaks have been reported

FIGURE 1

Number of *Campylobacter* and *Salmonella* case notifications and notification rates registered at the Federal Office of Public Health, Switzerland, 1988–2013



where fewer than 100 organisms had caused disease [11].

In Switzerland, *Campylobacter* replaced *Salmonella* as the most frequent food-borne pathogen isolated from clinical specimens in 1995 [12]. In Europe, predominance of *Campylobacter* has been reported from 2005 onwards [13]. *Campylobacter* notifications were stable in European Union (EU) countries between 2009 and 2013 while *Salmonella* notifications declined. Nonetheless, reported food-borne outbreaks were more often caused by *Salmonella* spp. than by *Campylobacter* spp. (1,168 vs 414 in 2013).

The aim of this study is to describe the epidemiological patterns and trends of *Campylobacter* and *Salmonella* case notifications in Switzerland and to identify factors leading to the inverse trends observed from the NNSID.

Methods

Medical diagnostic laboratories in Switzerland are obliged by law to report positive *Campylobacter* and *Salmonella* test results to the FOPH and to the cantonal chief medical officer in the patient's canton of residence within one week of discovery [1]. Reports must include information on laboratory diagnosis (test result, interpretation, type of sample, detection method and date), patient data (sex, date of birth and place of residence) and physician- and diagnosing laboratory-related data (name, phone and fax number, and address). The FOPH enters the information into the NNSID database. If the patient's canton of residence is unknown, the canton of the reporting laboratory is entered.

The present study used *Campylobacter* and enteric *Salmonella* case notification data from the present NNSID's first full year of data collection (1988) until the end of 2013. Data on patients residing outside of Switzerland were excluded. If residency was not specified, the record was kept in the analysis. Notification

rates, defined as the number of cases per 100,000 resident population, were calculated. The term 'notification rate' was used instead of 'incidence rate' to be consistent with other authors [13] and because the numbers calculated should not be equated with a true population incidence. To calculate notification rates, data on the average permanent resident population, obtained from the Federal Statistical Office's STAT-TAB database, were used [14]. Data was analysed and graphically represented using the statistical software Stata (Version 13.0).

Results

Campylobacteriosis trends

A 2.5-fold increase in the number of reported campylobacteriosis cases, from 3,127 cases in 1988 to 7,499 cases in 2013, was observed (Figure 1).

Case numbers increased steadily from 1988 to 2000, until they reached 7,000. Thereafter, *Campylobacter* case notifications dropped and levelled off at 5,000 cases annually and then rose steadily again from 2007, exceeding the peak level reached in 2000. The highest number of cases reported to date was 8,480 cases in 2012. In each year since 1988, a peak was observed during the summer months (June–August) (Figures 2 and 3).

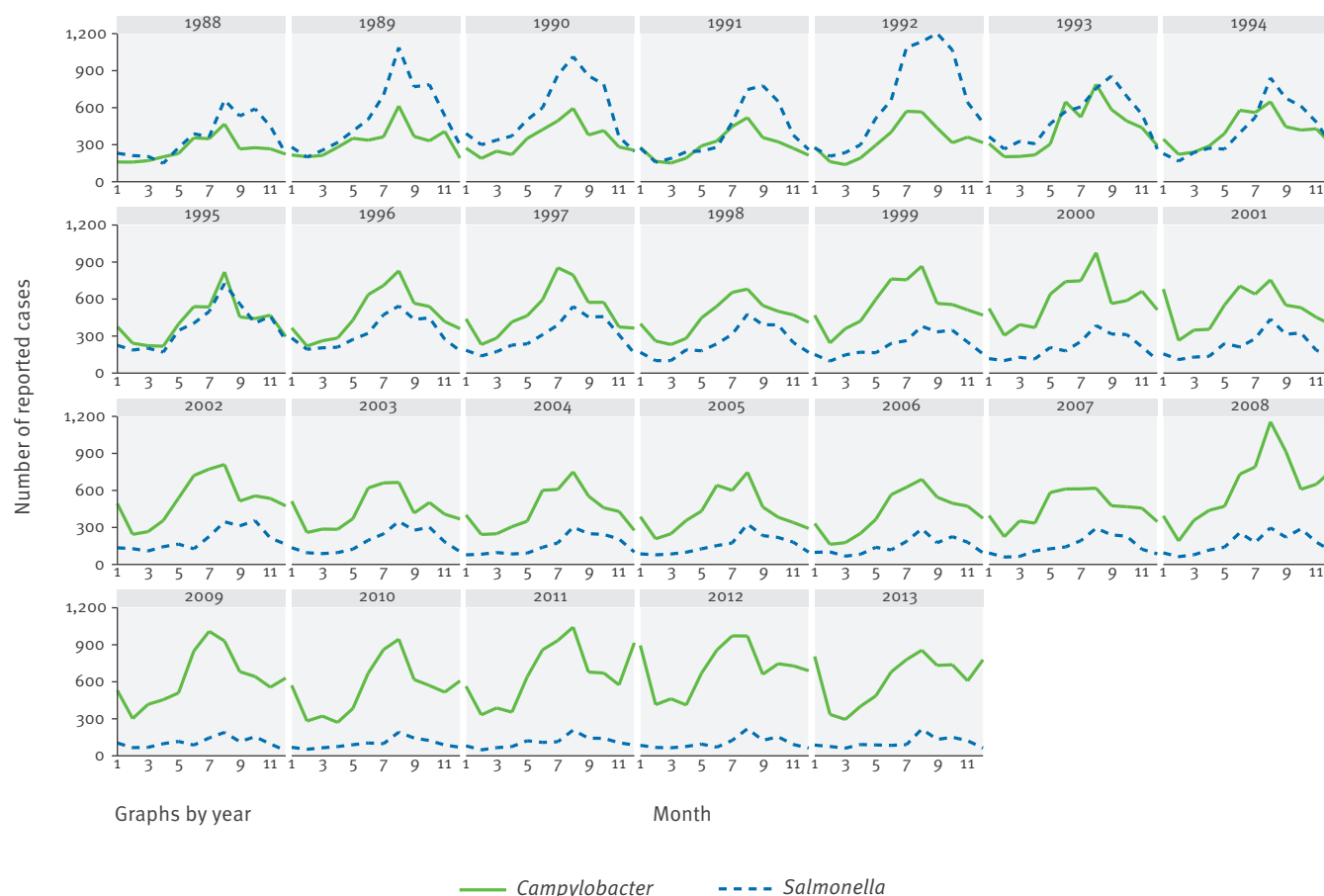
A second, much shorter peak was noted in December and January in all years. This winter peak has been especially pronounced in the past few years. While the highest weekly case numbers during the summer and winter peaks were comparable in 2009 and 2010, weekly case numbers were much higher during the winter peaks of 2011/12, 2012/13 and 2013/14 compared with the preceding summer peaks (Figure 3).

The increase in *Campylobacter* case notification rates differed by age (Figure 4). Among younger age groups, the increase in notification rates over the years was less pronounced than among older age groups. In children younger than five years old, the notification rates decreased from 105.3 to 102.3 cases per 100,000 population between 1988 and 2013 (-3%) (Table 1).

This decrease was statistically significant (permutation test for trend, $p=0.03$). There was no statistically significant (decreasing or increasing) trend in the 5–9 year-olds; in all older age groups, the increasing trend was statistically significant (permutation test for trend, $p=0.01$ for 20–24 year-olds, $p<0.01$ for all other age groups). Among those aged 85 years and older, the notification rate increased more than seven-fold, from 11.7 to 92.2 cases per 100,000 population during the same time period. The median age of campylobacteriosis patients increased from 25 years (interquartile range, IQR: 17–38) in 1988 to 39 years (IQR: 23–59) in 2013. In all but the 20–24 year-old age group, notification rates were higher for males than for females (Figure 4). Males accounted for 53.4–57.5% of total case notifications each year.

FIGURE 2

Monthly number of notified campylobacteriosis and salmonellosis cases, Switzerland, 1988–2013



Campylobacter diagnostics identified *C. jejuni* or *C. coli* in the majority of clinical samples (88.5–96.8% every year; data not shown). For most of the remaining cases, the species was not identified or not reported. Reported sample material came from stool (98.8%), blood or serum (0.4%), and other or unspecified materials (0.8%). The majority of cases were tested using culture-based methods directly or confirmatively after PCR (>97%).

Salmonellosis trends

Salmonellosis cases reported to the FOPH increased from 4,291 cases in 1988 to 7,806 cases in 1992 (Figure 1). Since 1992, *Salmonella* case notifications steadily decreased until reaching 1,267 cases in 2013. The highest number of *Salmonella* case notifications each year was registered in late summer (July–October), indicating a seasonal pattern (Figures 2 and 3).

Time trends did not differ between sex and age groups (Table 1, Figure 5).

Each year, 49.6–56.2% of reported cases occurred in males. The median age of salmonellosis patients increased from 25 years (IQR: 7–44) in 1988 to 29 years (IQR: 11–56) in 2013. In terms of notification rates, the highest absolute reduction occurred in the youngest

age group (under five years, Figure 5). The reduction was, however, similar for all age groups when looking at percentage decrease (Table 1). The decreasing trend for all age groups from 1988 to 2013 was statistically significant (permutation test for trend, $p < 0.01$ for all age groups).

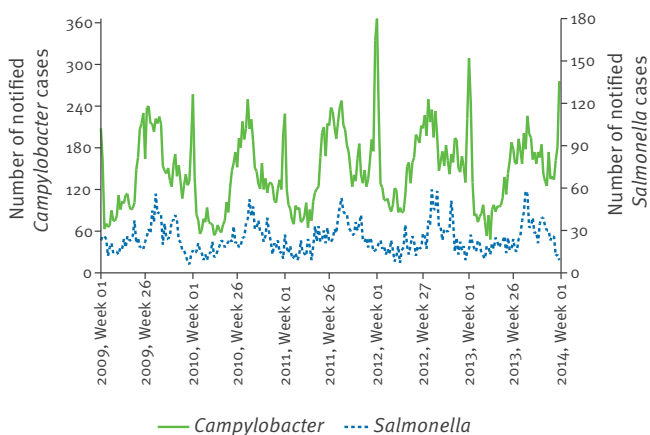
The two most frequently reported serovars were *S. Enteritidis* (54.0%) and *S. Typhimurium* (13.7%). Other reported *S. enterica* serovars included Virchow, Infantis and the monophasic Typhimurium 4,12:i:- (only differentiated in the notification system since 2010).

Discussion

In Switzerland, there has been a marked increase in *Campylobacter* case notifications since 1988, when surveillance began, while case numbers have decreased for salmonellosis. The number of *Campylobacter* infections nowadays is similar to levels of *Salmonella* 20 years ago. Salmonellosis has reduced considerably since then, due to control programmes targeting poultry production. Campylobacteriosis has also increased throughout the EU, though the numbers seem to have stabilised between 2009 and 2013; for salmonellosis, a decreasing trend continues [13]. Time trends for *Campylobacter* in Switzerland differ between age groups, even when looking at age-group-specific

FIGURE 3

Weekly number of notified campylobacteriosis and salmonellosis cases, Switzerland, 2009–2013



notification rates and adjusting for demographic changes in the population.

True increase in campylobacteriosis frequency

One study from the Netherlands showed that stool-testing frequency increased between 1998 and 2008, which might help to explain the increase in campylobacteriosis cases [15]. Along these lines, the decrease in salmonellosis cases would be even larger in the absence of intensified testing.

It is difficult to interpret the changes in the number of positive test results without knowing more about changes in the number of individuals seeking medical consultations, in the proportion of patients being prescribed stool testing and in the total number of tests performed (positive and negative) in Switzerland. Different factors can influence notification data such as changes in risk factors, in patients' health-seeking behaviour, in physician testing practices, in human susceptibility, or in the virulence or pathogenicity of *Campylobacter* spp. and *Salmonella* spp.

When a patient presents with acute gastroenteritis necessitating further laboratory testing, Swiss physicians most commonly request basic stool bacteriology, which includes testing for *Campylobacter*, *Salmonella* and *Shigella* (data not shown). Therefore, a change in testing frequency without a change in disease epidemiology would most likely lead to a similar change in both *Campylobacter* and *Salmonella* case notifications. Some improvements in stool culture methodology have been made in the past 25 years; however, changes cannot explain the inverse trends observed (personal communication, Roger Stephan, 30 July 2015). Furthermore, negative test results are not notifiable and, hence, the total number of tests (denominator) is unknown. Knowing the denominator would help to confirm or reject the hypothesis that a change in testing frequency does not explain the increase in *Campylobacter* case notifications and would allow for a better interpretation

of the trends observed in the NNSID. Though stool culture methods did not change significantly, the physicians' awareness towards campylobacteriosis is likely to have increased. It is not known to what extent this might have influenced notification data. Changes in patients' health-seeking behaviour are unlikely to influence *Campylobacter* and *Salmonella* case notifications in different ways. Consequently, we assume that the decrease in *Salmonella* case notifications and the increase in *Campylobacter* case notifications represent real epidemiological trends.

The revised Swiss Epidemics Act effective since January 2016, and its allocated ordinances obligates diagnostic laboratories to report annually the total number of positive and negative *Campylobacter* and *Salmonella* tests performed [16]. This innovation will allow basic routine analysis of trends in testing frequency and positivity rates in the future.

The influence of sex and age on food-borne disease notifications

Salmonella case notifications do not differ between sexes, even when stratified by age groups. In contrast, *Campylobacter* case notifications reveal higher notification rates among males in all age groups, except for those in the 20–24 year-old group. Interestingly, studies from Germany and England and Wales also show that females in their twenties are more frequently affected by campylobacteriosis than males, while male cases dominate in all other age groups [17,18]. Schielke et al. [17] suggested that women in this age group are more frequently involved in childcare activities, which might lead to increased human-to-human transmission. They also suggest that women in this age group are more often exposed to potentially contaminated chicken because they prepare and eat chicken more frequently than men of the same age. They may also be in closer contact with pets, which often harbour the same strains as their owners [19]. Different help-seeking behaviour of patients in this age group or different testing practices of physicians could also explain variations. Moreover, it seems likely that genetic or hormonal factors lead to differences by sex, as notification rates in males and females differ already in the youngest age group (under five years) (Figure 4) [20]. We assume that in the youngest age group, health-seeking behaviour and eating habits are not yet dependent on sex and are rather driven by parents or other persons engaged in childcare.

Available information from England and Wales also shows that adults, including the elderly, increasingly test positive for *Campylobacter* [18]. It has been suggested that the increasing use of proton pump inhibitors (PPIs) might explain a part of this phenomenon, especially among the elderly. Several studies have found that the use of PPIs is a risk factor for infection with *Campylobacter* and other enteric pathogens [21]. However, one study revealed that patients prescribed PPIs were already at increased risk of gastrointestinal

FIGURE 4

Trends in *Campylobacter* notification rates between age groups and sexes, Switzerland, 1988–2013



infection, even before prescription of these drugs [22]. In any case, conditions leading to PPI use or prescription are likely associated with acute infectious gastroenteritis. Why the aforementioned risk factor would only influence the frequency of *Campylobacter* but not *Salmonella* case notifications remains unknown. One possible explanation is that the infective dose of *Campylobacter* is generally lower than that of enteric *Salmonellae*. A recent study of poultry consumers' behaviour, risk perception and knowledge related to campylobacteriosis and domestic food safety showed that unsafe cooks were more likely to be male and of younger age [23]. Even though this finding is consistent with high *Campylobacter* notification rates observed among young adults, it does not explain the increasing rates among the elderly.

Food safety regulations

Campylobacter and *Salmonella* infections are assumed to be mainly food-borne. Genotyping and epidemiological studies in Switzerland have shown that chicken meat is the most likely source of infection in the majority of human campylobacteriosis cases [5,24–26]. In concert with these findings, a recent time-series analysis showed a significant association between *Campylobacter* prevalence in broiler chickens and human illness [27]. In Switzerland, poultry consumption

has increased in the past 25 years. While the average per capita consumption was 7.8 kg in 1988, it was 11.4 kg in 2013 [28,29].

Eggs and egg-containing products were shown to be risk factors for salmonellosis in Switzerland in 1993 [30]. The legislating authorities addressed the risk of these food-borne pathogens by setting and enforcing microbiological criteria.

As early as 1969, an official method to detect enteritic *Salmonella* in foods was published in the Swiss Food Manual [31]. Also, guidance levels for *Salmonella* in different food categories were given.

In 1981, legal microbiological criteria for foods were decreed for the first time in a Federal Ordinance [32]. Criteria for *Salmonella* were as follows. For baby foods and diet products: not detectable (nd) in 50 g; drinking water: nd in 5 l; other products: nd in 20 g. For 'other products', authorities could refrain from measures if the product in question had to undergo treatment (e.g. cooking) prior to consumption. In 1995, after a revision of the ordinance, criteria for *Salmonella* were set at as follows. For baby foods: nd in 50 g; drinking water: nd in 5 l; ready-to-eat foods: nd in 25 g; and spices: nd in 25 g [33]. In 2005, Swiss food legislation adopted

FIGURE 5

Trends in *Salmonella* notification rates between age groups and sexes, Switzerland 1988–2013



the European Union's microbiological criteria for *Salmonella* in food [34].

Salmonella limits for some categories of raw meat were issued as the national law adapted to EU hygiene regulations in 2006 [35]. To combat the epidemic with *S. Enteritidis* in eggs, mandatory screening of layer hens and measures to eradicate positive flocks were decreed by the Ordinance for the Control and Eradication of Epizootic Diseases as early as 1993 [36]. Apart from a ban on battery-caged chicken rearing (in effect since 1992 [37]), no further measures (such as vaccinations of layer hens against *S. Enteritidis*) are implemented in Switzerland.

As early as 1987, a limit for *Campylobacter* was decreed in the Ordinance on Hygiene, which was 'not detectable in 10 g of ready-to-eat foods' (later, not detectable in 25 g) after enrichment. This microbiological criterion was abrogated in 2006. To address the risk of *Campylobacter* in connection with poultry liver, since 2014 the Ordinance has stipulated that poultry liver must be sold frozen if it cannot be shown that the product comes from a *Campylobacter*-free flock [35]. Furthermore, a process hygiene criterion to minimise *Campylobacter* in poultry slaughterhouses is underway and should enter into force in 2016. However, criteria

for *Campylobacter* on raw poultry meat are not currently being considered.

Relevant epidemiological studies in Switzerland

In 2013, 37.7% (169/448) of broiler flocks and 65% (226/348) of rectum-anal swab samples taken from pigs at slaughter tested positive for *Campylobacter* [38]. In the same year, only 1% of 3,636 samples of fresh poultry meat, poultry meat preparations and poultry meat products at different stages of processing tested positive for *Salmonella*. Twenty-three years prior, *Salmonella* contamination levels in Switzerland were much higher. In a 1990 study, 19.2% of chicken neck skin lobes and 47.7% of broiler flocks were found to be *Salmonella*-positive [39]. As a consequence, *Salmonella* control measures as described above were implemented in the 1990s and led to a significant reduction in the number of human cases reported.

In Switzerland, salmonellosis and campylobacteriosis case curves crossed in 1995; in Austria, it was in 2006 [40]. The reason for this striking difference might be that Switzerland addressed the epidemic of *S. Enteritidis* in eggs at a very early stage.

The reduction of domestic salmonellosis cases resulted in a higher prominence of travel-associated

TABLE

Comparison of notification rates for *Campylobacter* and *Salmonella* among different age groups, Switzerland, 1988 and 2013

Age group	<i>Campylobacter</i>			<i>Salmonella</i>		
	Notification rate		%	Notification rate		%
	1988	2013	increase	1988	2013	increase
<5	105.3	102.3	-3%	216.1	51.5	-76%
5–9	49.9	62.9	+26%	85.1	23.4	-73%
10–14	29.7	58.1	+96%	59.1	15.1	-74%
15–19	54.7	108.1	+98%	63.4	18.1	-71%
20–24	97.4	160.7	+65%	68.1	25.3	-63%
25–44	49.2	91.2	+85%	51.6	10.6	-79%
45–64	24.4	78.3	+221%	41.1	10.9	-73%
65–84	19.2	100.1	+421%	38.6	15.1	-61%
85+	11.7	92.2	+688%	62.7	9.3	-85%

transmission risks in relative terms, which was shown by Schmid and Baumgartner: the (relative) proportion of travel-associated *S. Enteritidis* cases increased substantially from 20% in 1993 to 45% in 2011/12 [41]. Two case–control studies on campylobacteriosis [5,26] and a case–control study on salmonellosis [30] conducted in Switzerland identified travel abroad as a risk factor for the diseases. However, this finding has to be interpreted with care, as patients with travel history are more likely to be tested (data not shown) and all studies recruited laboratory-confirmed cases.

The observed winter peak in *Campylobacter* infections can be attributed partly to the traditional consumption of meat fondue over Christmas and New Year [5]. However, it is not known why this winter peak has been more pronounced in the past few years. Given the increasing per capita consumption of poultry meat [28,29], one could hypothesise that poultry has become more popular in meat fondues.

Outbreaks due to *Campylobacter* or *Salmonella* also occurred in Switzerland. However, the number of food-borne outbreaks decreased significantly between 1993 and 2010, mainly due to the reduction of salmonellosis [12]. The number of registered *Salmonella* outbreaks dropped from 27 in 1993 to one in 2010 while the number of *Campylobacter* outbreaks varied between none and five throughout this time period. In relation to the number of cases, *Salmonella* is causing more outbreaks than *Campylobacter* both in Europe and in Switzerland.

Public awareness and knowledge about the diseases

Public awareness and people's knowledge of *Campylobacter* and *Salmonella* in Switzerland are as different as the trends observed in the two pathogens in the NNSID. In 2011, a consumer survey showed that 76% of participants were 'very concerned' or 'somewhat concerned' about *Salmonella* in foods [42]. Only

1% of respondents stated that they had not heard of the *Salmonella* bacterium. In contrast, only 33% were 'very concerned' or 'somewhat concerned' about *Campylobacter* and more than half (52%) had not heard of the pathogen. Unpublished data from a recent case–control study in Switzerland [5] confirm those figures: 55% of people infected with campylobacteriosis (cases) and 68% of healthy people (population-based controls) had never heard of *Campylobacter*, while only 2% of cases and 3% of controls had never heard of *Salmonella*.

The lack of knowledge about safe food handling and avoidance of cross-contamination, and low personal risk perception are the main reasons for unsafe food handling [23,43]. The high prevalence of *Campylobacter* in chicken products, the low infective dose of *Campylobacter* and the increasing consumption of chicken meat combined with the apparent lack of knowledge about the *Campylobacter*-pathogen are all factors facilitating infection.

Conclusions

Campylobacter spp. infections are a serious and increasing public health concern in Switzerland. For *Salmonella* spp. infections, an epidemiological turnaround has been achieved through concerted efforts and legal regulations of the poultry- and food-production industries, but little has been done to date to prevent *Campylobacter* infections on a large scale. Food safety interventions before the sale of poultry meat are urgently required to reduce *Campylobacter* contamination frequencies. Since the number of control options is limited, the hygienic treatment of chicken carcasses with chemicals, for example peracetic acid, should not be excluded from discussion [44]. However, the population's limited awareness of *Campylobacter* must also be addressed. It seems reasonable to believe that the same type of behaviour changes that reduced *Salmonella* infections can be applied to prevent *Campylobacter* infections and that caution can be extended from eggs to raw poultry meat, cutting boards and knives.

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Conflict of interests

The authors declare that they have no conflict of interest.

Authors' contributions

DM and MM conceived the idea of and designed the study with CS; MJ provided the data; CS and DM analysed the data and CS wrote the first draft; MJ, AB, MM and DM contributed to the interpretation of the data, writing and reviewing of the manuscript.

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Early influenza vaccine effectiveness results 2015-16: I-MOVE multicentre case-control study

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On 11 February 2016, the Influenza Monitoring Vaccine Effectiveness in Europe (I-MOVE) published the 2015–16 interim vaccine effectiveness (VE) estimates against influenza from a multi-centre case control study in 10 study sites: Germany, France, Hungary, Ireland, Italy, Poland, Portugal, Spain, Sweden and the Netherlands, on their website [1].

Adjusted VE interim results against any influenza among all ages were at 46.3% (95% confidence interval (CI): 4.9–69.7%) and 45.2% (95% CI: -12.5–73.3%) among the 18–64 year olds. Among those aged 65 years and older, there were only 14 influenza cases in the study. The adjusted VE against influenza A(H1N1)pdm09 was at 44.2% (95% CI: -3.1–69.8%) among all ages and thus lower compared with end of season estimates published in previous years (55.5% in 2010–11, 50.4% in 2012–13; 47.5% in 2013–14, 54.2% in 2014–15).

Early season influenza VE was measured against medically-attended laboratory-confirmed influenza from week 41/2015 to week 3/2016 using a test-negative design as described in the I-MOVE generic protocol [2] and in the I-MOVE multicentre case–control publications [3]. Some 1,933 influenza-like illness patients among whom 348 were positive to influenza were included: four cases of influenza A not subtyped, 246 A(H1N1)pdm09, 21 A(H3N2), and 77 influenza B cases. Among the 37 influenza B cases where lineage was available, 36 (97.3%) were of the Victoria lineage, a lineage not included in the trivalent vaccine.

For this interim analysis, there was no information on genetic characterisation of the viruses. The recently published European Centre for Disease Prevention and Control risk assessment [4] reported that all A(H1N1)pdm09 viruses characterised in the European Union up to week three belonged to the 6B subgroup.

The interim estimates should be interpreted with caution. The 2015–16 season started late in the participating countries and the sample size for these interim estimates is low, resulting in low precision. The final estimates will be available at the end of the influenza season.

Read more [here](#).

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European Commission Horizon 2020 programme call for vaccine development research into malaria and neglected infectious diseases, including Zika virus

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On 29 January 2016, the European Union (EU) Commissioner for Research, Science and Innovation, Carlos Moedas, informed that the EU would make available an additional EUR 10 million for urgent research on the Zika virus in response to the signals of an association between Zika virus infections and congenital abnormalities, including microcephaly [1]. This is in addition to EUR 40 million earmarked for research on vaccine development for malaria and neglected diseases, which includes the Zika virus, in the 'Call - Personalised Medicine' under the EU Horizon 2020 Work Programme 2016–2017 on health, demographic change and well-being [2]. The deadline for this call, is 13 April 2016 [2]. Its scope is to address bottlenecks in the discovery, preclinical and early clinical development of new vaccine candidates (antigens/adjuvants) for malaria and or neglected infectious diseases. Neglected infectious diseases for the scope of the call are, in addition to the 17 neglected tropical diseases prioritised by the World Health Organization [3] neglected viral emerging epidemic diseases, such as Zika virus disease, and childhood diarrhoeal diseases [2].

The expected impact of the call is:

- Proposals should deliver new vaccine candidates or move existing ones along the vaccine candidate pipeline to end by 2030 the epidemics of malaria and neglected tropical disease.
- To provide a reduction in the cost associated with late stage vaccine failure, increase the number of other vaccine candidates which can be tested with the same resources, and therefore increase the chance of discovery of an effective vaccine.
- If appropriate within the context of the European and Developing Countries Clinical Trials Partnership,

increase the number and quality of vaccine candidates for malaria and neglected infectious diseases available to proceed into further development and clinical testing.

The additional EUR 10 million for research infrastructures is aimed at contributing to the control of vector-borne diseases under the call 'Integrating Activities for Advanced Communities' [4]. The deadline for the call is 30 March 2016.

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International Committee of Medical Journal Editors seeks feedback on suggested requirements for sharing clinical trial data

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In a recent editorial, ‘Sharing Clinical Trial Data: A proposal from the International Committee of Medical Journal Editors’, the International Committee of Medical Journal Editors (ICMJE) puts forward that ‘As a condition of consideration for publication of a clinical trial report in our member journals, the ICMJE proposes to require authors to share with others the deidentified individual-patient data underlying the results presented in the article (including tables, figures, and appendices or supplementary material) no later than 6 months after publication’ [1].

The data sharing requirement is suggested to enter into effect for trials that start to enrol participants one year after the ICMJE requirements are adopted. Feedback following the proposals in the editorial will be considered before adopting the requirements.

The deadline for feedback via the [ICMJE website](#) is 18 April.

Read about the proposals of the ICMJE [here](#).

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