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The primary aim of the Danish enterovirus (EV) surveillance system is to document absence of poliovirus infection. The conflict in Syria has left many children unvaccinated and movement from areas with polio cases to Europe calls for increased awareness to detect and respond to virus-transmission in a timely manner. We evaluate the national EV laboratory surveillance, to generate recommendations for system strengthening. The system was analysed for completeness of viral typing analysis and clinical information and timeliness of specimen collection, laboratory results and reporting of clinical information. Of 23,720 specimens screened, 2,202 (9.3%) were EV-positive. Submission of cerebrospinal fluid and faecal specimens from primary diagnostic laboratories was 79.5% complete (845/1,063), and varied by laboratory and patient age. EV genotypes were determined in 68.5% (979/1,430) of laboratory-confirmed cases, clinical information was available for 63.1% (903/1,430). Primary diagnostic results were available after a median of 1.4 days, typing results after 17 days, detailed clinical information after 33 days. The large number of samples typed demonstrated continued monitoring of EV-circulation in Denmark. The system could be strengthened by increasing the collection of supplementary faecal specimens, improving communication with primary diagnostic laboratories, adapting the laboratory typing methodology and collecting clinical information with electronic forms.

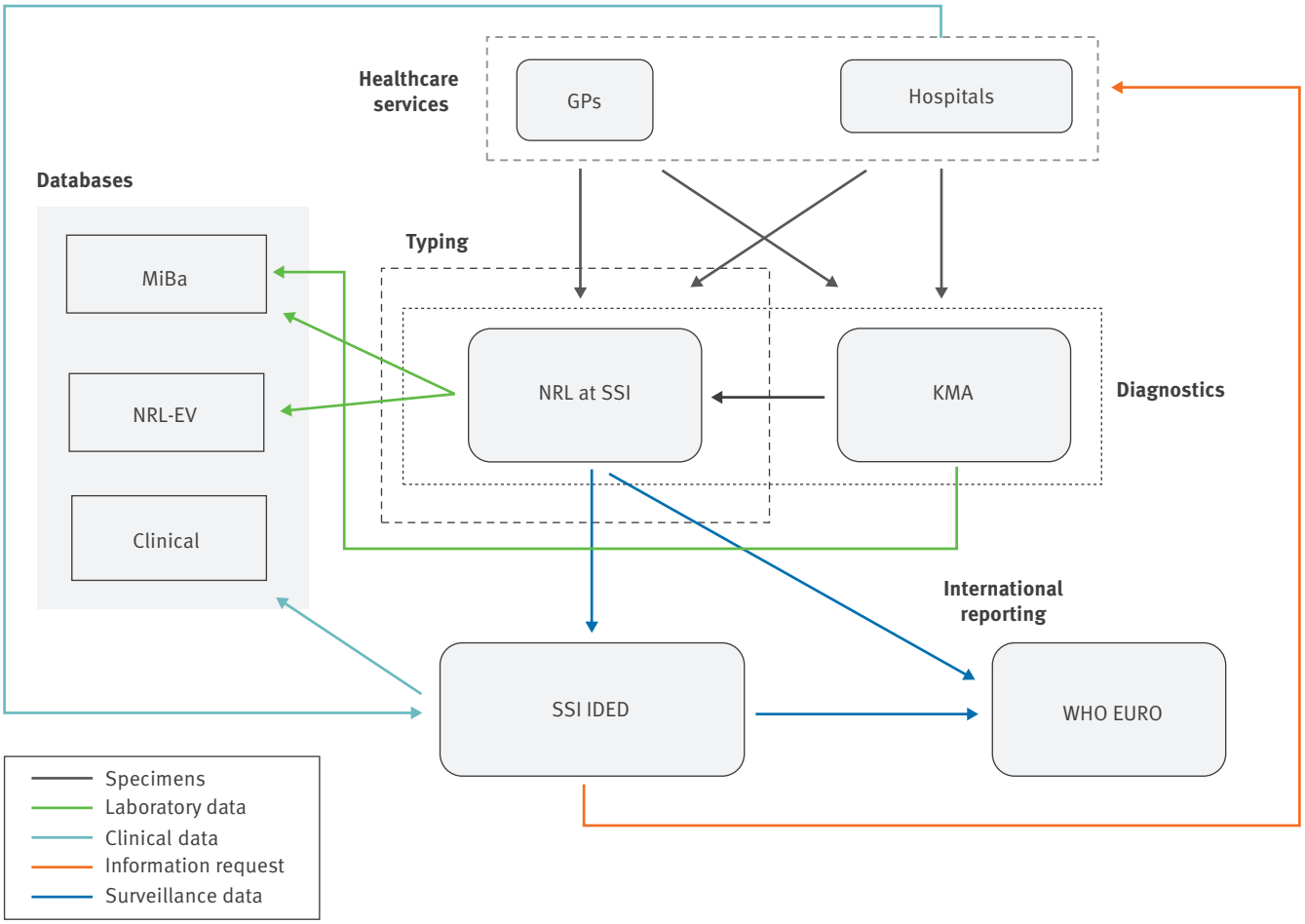
Introduction

Human enteroviruses (EV) is a diverse group of single-stranded RNA-viruses from the *Enterovirus* genus of the Picornaviridae family that includes polioviruses. A number of EVs are among the most common viral infectious agents in humans, with the majority of infections being asymptomatic or mild [1]. However, infection with EV can lead to a wide spectrum of symptoms including; upper respiratory illness, febrile rash, aseptic meningitis, pleurodynia, encephalitis, neonatal sepsis-like disease and acute flaccid paralysis (AFP) which may indicate poliomyelitis [1-3].

The World Health Organisation (WHO) announced the European Region as poliovirus free in 2002 [4]. In 2013 when only three countries had endemic circulation of poliovirus, namely Pakistan, Afghanistan and Nigeria, circulation of wild-type poliovirus was detected in three new regions: Syria, the Horn of Africa, and Israel [5-7]. Due to this recent transmission of poliovirus combined-+ with the armed conflict in Syria and the movement of refugees from this region, there is an increased risk for importation of poliovirus into Europe [7,8]. Considering this risk, there are currently concerns in the European Union (EU) and European Economic Area (EEA) over the quality of EV surveillance, in particular the capacity of countries to detect and respond to poliovirus transmission in a timely manner [9,10]. The European Centre for Disease Prevention and Control (ECDC) therefore advised in a 2014 technical report, entitled 'Detection and control of poliovirus transmission in the European Union and European Economic

Figure 1

Flowchart of the laboratory surveillance system for enterovirus in Denmark, routes of specimen flow, clinical information collection, databases and international reporting.



EV: enterovirus; GPs: general practitioners; IDED: Department of Infectious Disease Epidemiology; KMA: hospital clinical microbiological laboratory; MiBa: the Danish national microbiological database; NRL; Danish National WHO Reference Laboratory for poliovirus; SSI: Statens Serum Institut; WHO EURO: World Health Organization Regional office for Europe

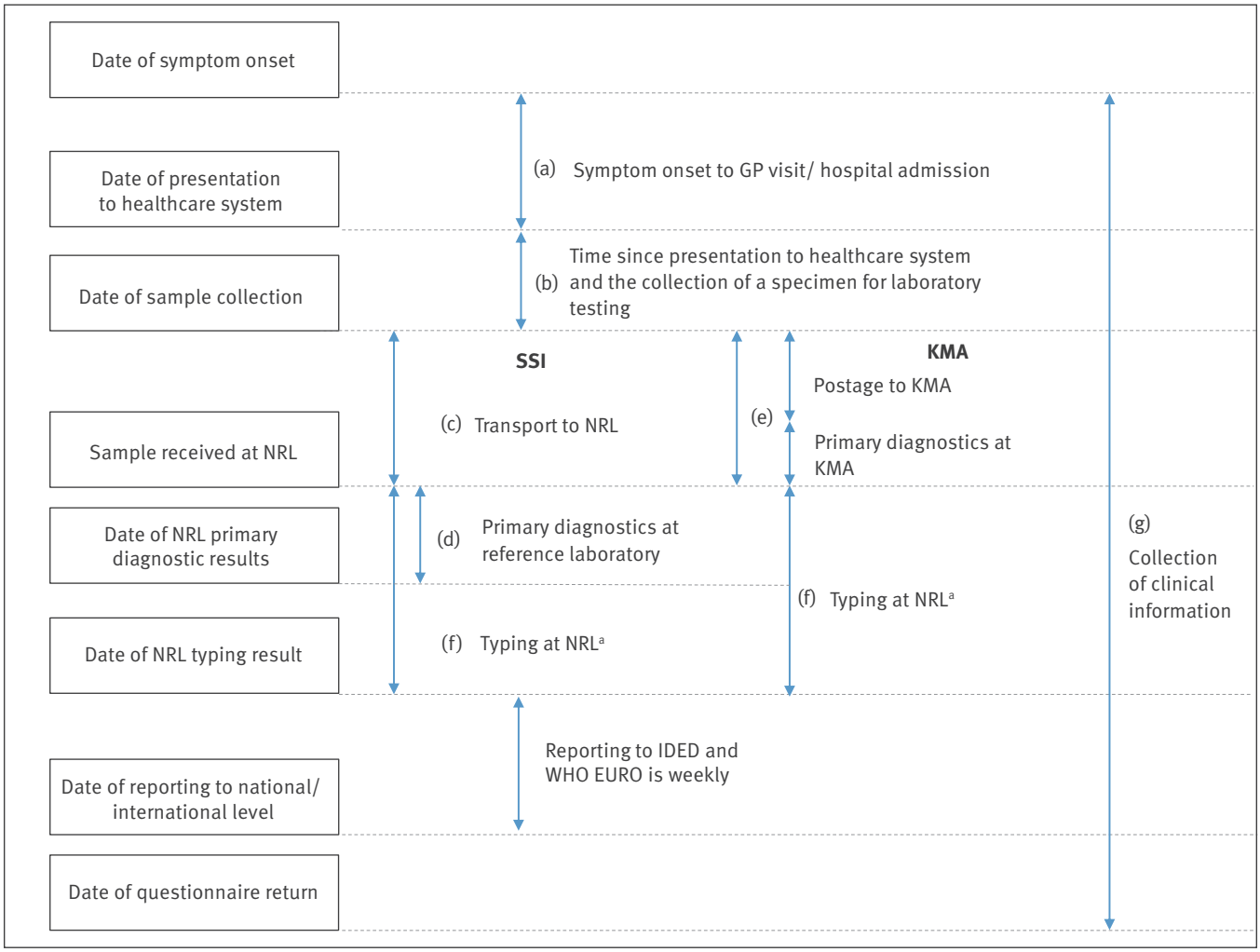
Specimens are sent for primary diagnostics to a KMA or directly to SSI. All data from the KMA and SSI are entered MiBa, all data from SSI is also entered into the NRL-EV database. The NRL reports new EV cases to the IDED. Reporting was monthly up until 2011 and weekly from 2012. The SSI IDED collects detailed clinical information with a letter and standardised questionnaire sent to the patient's hospital or GP. Returned questionnaires are entered into the clinical database by the IDED. Reporting to WHO EURO is carried out on a weekly from the reference laboratory; information is submitted for one sample per patient per day of sample collection. Reporting to WHO-EURO is yearly from the IDED.

Area', EU/EEA countries should assess the quality of their poliovirus surveillance and determine whether it needs to be strengthened [9]. A surveillance system allowing for rapid detection and a short time between specimen collection and outbreak response, would permit reducing transmission faster, while a slower response would conversely be associated with more widespread-transmission and therefore a greater cost and effort of containment [11]. This project aimed to address these points with regards to aspects of poliovirus surveillance currently operating in Denmark; the laboratory EV surveillance system and collection of detailed EV clinical information.

Poliomyelitis is a mandatorily notifiable disease in Denmark and a laboratory surveillance system based at the Danish National WHO Reference Laboratory for poliovirus (NRL) at Statens Serum Institut (SSI), in collaboration with the Danish clinical microbiology laboratories (Klinisk Mikrobiologisk Afdeling (KMA)), ensures year-round surveillance of all cases of EV-positive aseptic meningitis. The laboratory surveillance system is case-based and covers the entire population of Denmark; including all general practitioners (GPs), all hospitals and all KMAs. The system was initially set up as a component of the national poliomyelitis eradication plan, with the main objective of documenting the absence of poliovirus transmission in Denmark. This surveillance system collects data on EV to the genotype

Figure 2

Time intervals in the reporting of laboratory confirmed enterovirus cases in Denmark



EV: enterovirus; GP: general practitioner; IDED: Department of Infectious Disease Epidemiology; KMA: hospital clinical microbiological laboratory; NRL: Danish National WHO Reference Laboratory for poliovirus; SSI: Statens Serum Institut; WHO EURO: WHO Regional office for Europe.

[a]Could be calculated for 2013 only, relevant dates were not available before 2013.

The timeliness of interval (a) and (b) were calculated from the clinical database. For specimens where primary diagnostic was carried out at SSI, the intervals (c) and (d) were calculated from the NRL-EV database. For specimens where primary diagnostic was carried out at a KMA (e) was calculated from the NRL-EV database. For all samples subject to typing analysis the time interval (f) was calculated from the BioNumerics database and files containing sequencing run history, and was the time interval between arrival at the reference laboratory and the date when the first sequence result was obtained. The final time interval, (g) symptom onset to reporting of clinical symptoms to the department of epidemiology was calculated from the clinical database

level, as well as patient demographic information and clinical details; including symptoms, presentation with AFP and date of symptom onset. With this information the system also serves the objective to monitor national trends in circulating EV.

The surveillance system consists only of EV surveillance, is run on a voluntary basis and involves components that are passive, and there are consequently concerns with regards to underreporting. It has never been evaluated, and the completeness and timeliness are unknown.

The overall aims of the study were to describe the EV surveillance system and to determine whether it meets its surveillance objectives. In order to achieve this, we assessed the surveillance system and its characteristics, described the data sources, data providers, flow of diagnostic specimens, and mapped the routes of reporting to the national level and to WHO Regional office for Europe (WHO EURO). Hereafter, we evaluated the EV surveillance system for the attributes completeness and timeliness and made recommendations for improvements to the current system with regards to

firstly, documenting the absence of poliovirus transmission and secondly monitoring national trends in EV circulation.

Methods

Routes of reporting

The Danish EV surveillance system is run collaboratively by the NRL at the Department of Microbiological Diagnostics and Virology (MDV), SSI and the Department of Infectious Disease Epidemiology (IDED), at SSI. Although the system is voluntary it is highly recommended that EV-positive faecal or cerebrospinal fluid (CSF) specimens from patients with aseptic meningitis are sent to SSI. Between July 2014 and January 2015, interviews were conducted with staff of the NRL, IDED and the WHO EURO group lead for the Regional Laboratory Networks. Questions were asked in regards to the laboratory practices; the diagnostic and genotyping workflow and the surveillance system infrastructure; data sources, analysis and outputs. Finally, questions were asked about the system routes of reporting.

Laboratory identification and typing of enteroviruses

Interviews were additionally conducted in November 2014 with section chiefs or laboratory personnel responsible for EV testing, at the primary diagnostic level, at the KMAs. All 11 of the national KMAs were invited to take part in the study, either by telephone or by a paper-based questionnaire. Seven of the eleven KMAs took part in the interviews.

Four KMAs did not respond to the invitation for a telephone interview and did not return the paper questionnaire. Three of these KMAs: Mid-Vest, Esbjerg and Vejle did not test for EV during the study period. Only one of the non-respondent KMAs, Odense, did test for EV during the study period.

Questions were asked to ascertain whether the laboratories carried out EV testing and if so by what techniques. Additionally, KMAs were asked what criteria they followed for selecting specimens for forwarding to SSI.

Evaluation the surveillance system

The CDC 'Updated guidelines for evaluating a public health surveillance system' were used as a framework for the evaluation [12]. Completeness and timeliness were chosen as surveillance performance indicators for the analysis.

Data sources

Several central data sources were accessed for this evaluation and are outlined in Figure 1. Briefly, specimens are sent for primary diagnostics to a KMA or directly to SSI. Not all KMAs have the capacity to test for EV therefore specimens for EV testing may be forwarded directly to SSI or to another KMA. If a specimen is found EV-positive at a KMA it can be sent to the NRL for viral typing. Laboratory data for all EV primary diagnostic testing are contained in the Danish national microbiological database (MiBa). An extract was obtained from MiBa for all EV-positive serological, culture and RNA-based diagnostic tests and their results from 2010 to 2013. Details relating to laboratory testing carried out by the NRL are stored in the SSI laboratory information and management system (LIMS). All entries coded as EV culture, diagnostic PCR, and typing PCR were extracted from LIMS for the period of 01 January 2010 to 31 December 2013. This dataset is called the NRL-EV database herein.

Information on sequencing results for all EV-positive specimens typed at SSI for the corresponding study period was obtained in from an extract of the NRL sequencing results database, which is maintained with BioNumerics software and herein called the NRL-sequencing database.

The NRL reports new EV cases to the IDED, who in turn collects clinical information with a letter and standardised paper-based questionnaire sent to the patient's hospital or GP, and collects details on patient's symptoms in particular neurological symptoms including acute flaccid paralysis. The letter also reminds clinicians to send faecal samples from the patient to the NRL for characterisation.

The collected clinical data are stored in a database internally at the IDED. All entries in this clinical information database corresponding to the years 2010 to 2013 were extracted.

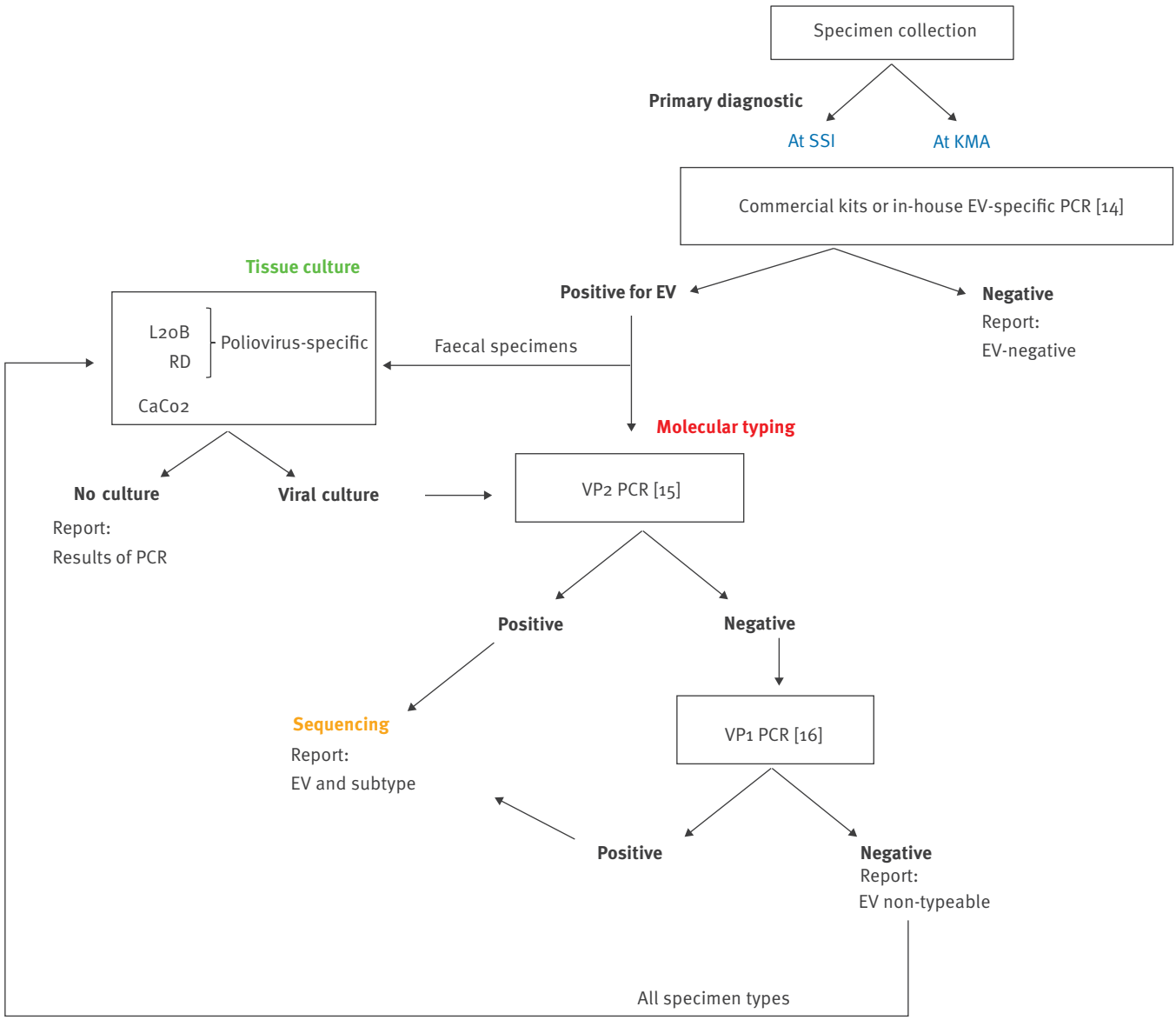
Data linkage between the various databases was possible and efficient due to the use of the Danish civil registry number (CPR numbers) as a unique identifier. This number is used in all four databases.

Completeness of the surveillance system

Completeness was assessed for the first component of the laboratory-based surveillance system; the forwarding of EV-positive specimens from primary diagnostic facilities to the NRL. All specimens that tested EV-positive on primary diagnosis, present in the MiBa database, were overlapped with the NRL-EV dataset, containing those specimens forwarded to SSI. If multiple specimens were taken from the same patient only one overlapping specimen was needed for all specimens associated with the patient to be considered overlapping. Samples were considered overlapping if there was no more than a 14 day difference between the sampling dates indicated in the two databases. If more than one specimen was collected from the same patient on the same day, only one was counted in the analysis. The proportion of specimens positive in MiBa present in the NRL-EV dataset was calculated as the performance indicator. This calculation was performed for CSF and faecal specimen types only, and repeated for all specimen types that were submitted

Figure 3

Flowchart of the laboratory diagnostic workflow, for the surveillance of enterovirus in Denmark



CSF: cerebrospinal fluid; EV: enterovirus; KMA: hospital clinical microbiological laboratory; SSI: Statens Serum Institut

KMAs differ in their methods for primary diagnostics. At SSI primary diagnostics is carried out with a multiplex real-time PCR. A reverse-transcriptase PCR targeting the VP2 capsid protein is the primary EV typing assay. If VP2 typing fails, a reverse-transcriptase semi-nested PCR targeting the VP1 capsid protein is used. PCR amplicons are sequenced. All faecal specimens, as well as CSF and other specimens where PCR has failed are subject to viral cultivation assays. Specimens are cultured in three cell lines, two poliovirus specific cell lines; L20 and RD and one cell line for the non-specific cultivation of EV, CaCO2. Positive viral cultures are then subject to the EV typing workflow. If a typing result was obtained from either VP2 or VP1 the results are reported as EV and the genotype is given. If no product is obtained from either assay the isolate is reported as EV non-typeable. If poliovirus is detected from the cultivation process, further characterisation based on virus neutralisation as well as RT-PCR will be applied to determine the poliovirus type as well as to discriminate between wild-type or vaccine-derived poliovirus, respectively.

for EV surveillance; faecal samples, CFS, blood, serum, plasma, biopsy, swabs, bronchoalveolar lavage, expectorate, naso-pharyngeal secretions, pulmonary secretions, pericardial fluid, pus, saliva and urine. Results were stratified by primary diagnostic laboratory, patient age and analysed for statistical differences using a Chi-squared test. The completeness over the test period was analysed for trends using Poisson regression. Binomial proportion 95% confidence intervals (CIs) were calculated.

Secondly, the completeness of the typing analysis carried out at the NRL was determined. A dataset extracted from the NRL-EV database corresponding to all samples that were EV-positive was overlapped to the NRL-sequencing database, containing typing results. The

Table 1

Completeness of EV-positive CSF and faecal specimen forwarding from primary diagnostic laboratories to the National Reference Laboratory; by year, patient age and primary diagnostic laboratory, Denmark, 2010–2013 (n=2,202)

	Positive specimens[a]	Sent to reference laboratory	Not sent to reference laboratory[b]	Percentage Completeness (%)	95% Confidence Intervals
All sample types					
Total number of specimens	2,202	1,712	490	77.7	75.9-79.4
CSF and faecal specimens					
Total number of specimens	1,063	845	218	79.5	76.9-81.8
KMA of primary diagnostic[c]					
Aalborg	22	10	12	45.5	26.9-65.3
Herlev/ Hillerød/ Hvidovre	103	45	58	43.7	34.5-53.3
Odense	216	203	13	94.0	89.9-96.4
Region Sjælland	15	11	4	73.3	48.1-89.1
Rigshospitalet	70	39	31	55.7	44.1-66.7
SSI[d]	377	377	0	100	98.9-100
Aarhus	249	151	98	60.6[e]	54.5-66.5
Sønderborg	11	9	2	81.8	52.3-94.8
Patient age[c]					
Less than one year	293	258	35	88.1	83.8-91.2
Aged 1 year or older	770	587	183	76.2	73.1-79.1
Year					
2010	227	185	42	81.5	75.9-86.0
2011	314	267	47	85.1	80.6-88.6
2012	305	244	61	80.0	75.1-84.1
2013	217	149	68	68.7	62.2-74.4

KMA: hospital clinical microbiological laboratory; SSI: Statens Serum Institut.

[a]The calculation counts one specimen per patient per 14 days (specimens taken within a 14-day period are considered to be relating to the same episode of illness).

[b]This value reflects different practices in the KMAs, including differences in the selection criteria for sending specimens where low volume remains following the KMA's primary diagnostic workflow.

[c]Indicates groups where completeness values were statistically different between strata

[d]The SSI primary diagnostic laboratory and the NRL are located in the same building

[e]All specimens are checked, and only those with sufficient material are sent to the NRL.

performance indicator was the number of EV-positive specimens subject to typing analysis over the total number of EV-positives. The completeness of the genotype result was also determined; and the performance indicator was the total number of specimens where a subtype could be determined over the total number of EV-positive specimens.

Thirdly, completeness of the clinical data (e.g. symptoms of CNS affection, paralysis etc) for EV-positive cases was determined by comparing the number of patients with clinical information available to the number of patients with no clinical information available. An extract was made of the NRL-EV dataset containing all EV-positive cases. This was overlapped with the clinical information database. The performance indicator was the number of EV-positive cases where detailed clinical information was collected over the total number of EV-positive cases. The results were stratified for patient age and analysed for statistical differences using a Chi-squared test. The completeness by year was analysed for a trend with Poisson regression. Binomial proportion 95% CIs were calculated.

Timeliness of the surveillance system

The time intervals between six steps in the surveillance system were evaluated using various time variables from the four databases described above. The six steps (a) to (g) are outlined in Figure 2. Intervals for steps (a) to (e) and (g) were calculated for the years 2010 to 2013, intervals for step (f) could be calculated for 2013 only, dates were unavailable for the previous years. The time intervals were calculated in days. For each time interval the median, 25% and 75% quartiles and the interquartile range (IQR) were calculated using STATA V12.1 software. The timeliness calculations were stratified for patient age, geographical location, hospital of patient origin, laboratory of primary diagnostic and laboratory result. Strata were compared for differences with a one-way analysis of variance. Trends in the timeliness values over the test period were analysed with linear regression.

Table 2

Completeness of patient clinical information for enterovirus-positive specimens, by year and age for all sample types, Denmark, 2010–2013 (n=1,430)

	Total no. positive specimens	Total no. clinical information collected	Total no. clinical information not collected	Percentage completeness (%)	95% Confidence Intervals
All cases	1,430	933	497	65.1	62.7-67.7
Year					
2010	318	270	48	84.9	80.6-88.4
2011	285	92	193	32.3	27.1-37.9
2012	452	263	189	58.2	53.6-62.7
2013	375	308	67	82.1	77.9-85.7
Patient age[a]					
Less than one year of age	948	605	343	63.8	60.7-66.8
Aged 1 year or older	482	328	154	68.0	63.8-72.1

[a]Indicates groups where completeness values were statistically different between strata

Surveillance standards

Surveillance standards were based on those for AFP along with the objectives of the EV surveillance system [13,14]. The following surveillance standards were considered acceptable for the system:

≥80% of positive CSF or faecal specimens, determined on primary diagnostics should be submitted to the NRL for viral typing;

- ≥80% of positive CSF or faecal specimens, should arrive at the NRL within 7 days of specimen collection;
- the final laboratory typing result should be available for≥80% of specimens within 28 days of sample arrival;
- clinical information should be collected for≥80% of cases with a positive specimen sample.

Results

Surveillance system structure and laboratory workflow

From the interviews the structure of the system, including specimen flow, data flow, databases and routes of reporting was elucidated (Figure 1).

At the six KMAs that were interviewed, the EV primary diagnostics differed in terms of assays used and range of sample material they had capacity to test. One of the interviewed KMAs did not test for EV, four of the KMAs used a commercial, automated real-time multiplex reverse transcription-PCR for the diagnosis of EV from CSF, but did not test other sample materials. One KMA used an in-house PCR for all sample materials. In all cases KMAs sent positive CSF samples to SSI and supplementary faecal samples from the positive patients should also be sent to SSI. Interviews elucidated different practices locally at the KMAs with regards to criteria for sending low-volume CSF specimens to the NRL. Some KMAs sent all specimens including low volume whereas others did not. These differences impacted on the completeness values.

Specimens from all regions of Denmark were sent to SSI for primary diagnostics. Primary diagnosis at SSI was carried out with a one-step multiplex real-time PCR as described by Nielsen et al. in 2013 [15].

VP2 and VP1 regions of EV-positive specimens were PCR amplified, as described by Nasri et al. and Nix et al., respectively [16,17]. Amplicons from these PCRs were sequenced and genotypes obtained by sequence comparisons within the NRL-sequencing database, and the NCBI, using the BLAST software. Genotyping was carried out at the NRL only. The laboratory workflow for EV typing at SSI is outlined in Figure 3.

Surveillance data

During the study period a total of 23,720 samples were tested for EV in Denmark; 9.3% (2,202/23,720) were positive. Of these samples 16,538 (931 positive samples) were tested at KMAs while 7,182 (1,271 positive samples) were submitted directly for testing at SSI.

A total of 10,945 CSF specimens were tested, 7.7% (844/10,945) were EV-positive; of 2,211 tested faecal specimens, 9.9% (219/2,211) were EV-positive; 17.6% (149/844) positive CSF specimens had a faecal submitted for analysis also. The remaining 10,564 samples, comprised blood, serum, plasma, biopsies, swabs, pulmonary secretions, pericardial fluid, pus,

saliva and urine and unknown sample types and 10.4% (1,106/10,564) were EV-positive.

During VP2 and VP1 amplification and subsequent typing and alignment, no poliovirus (wild-type or vaccine-derived) was detected.

Completeness

Submission of EV-positive specimens to the National Reference Laboratory

The completeness of submission of EV-positive specimens to NRL for further characterisation was 77.7% for all specimen types (1,712/ 2,202 positive specimens) (Table 1). For CSF or faecal specimens, completeness of 79.5% was obtained (834/1,063 positive specimens) (Table 1).

There was no increasing or decreasing trend in the numbers of CSF or faecal specimens forwarded to the NRL over the four years). However, the completeness varied by year and was above the adopted surveillance standard cut-off of ≥ 80% in three of the years and was below the cut-off in 2013 only (Table 1). The level of completeness also varied according to the location of primary diagnostics; the KMA of sample origin, (p-value < 0.001), and was above the surveillance standard of ≥ 80% for three KMAs (Table 1). Some KMAs check the samples for sufficient material before sending them to the NRL and withhold samples that would not be processed further by the NRL. Thus, the number of EV-positive specimens evaluated by the KMAs is higher than those actually sent to the NRL. The completeness of this step for all samples, including those with too little sample material remaining, was significantly higher, and above the surveillance standard threshold, for patients aged one year or younger compared with those older than one year, (p-value < 0.001) (Table 1).

EV genotyping carried out at the National Reference Laboratory

There were 1,430 EV-positive specimens in the NRL-EV database; 863 following primary diagnostic at SSI and 567 from KMAs. Of these, 1,344 were genotyped, 86 specimens were not genotyped because they lacked sufficient sample material. All samples where sufficient material remained following primary diagnosis were subject to genotyping. The completeness of this component of the surveillance system was therefore 94.0% (1,344/1,430). Of these, no amplicon could be obtained with the genotyping PCR for a total of 365 specimens; they were therefore reported as non-typeable EV. The completeness of a final genotyping result was therefore 68.5% (979/1,430).

Detailed clinical information for entereovirus-positive cases

Completeness of detailed clinical information for EV-positive cases over the four year period was determined as 65.2% (Table 2). Although there was no trend in the completeness detected over the four year period, the completeness of this step varied throughout the years; two years 2010 and 2013 were above the surveillance standard while the values for 2011 and 2012 were well below the standard (Table 2). The collection of detailed clinical information was not different for cases over and under one year of age.

Timeliness

The median timeliness of the six steps in the surveillance system is summarised in Table 3. The median for the first interval symptom onset and presentation to the health system was significantly shortened for cases under 1 year of age; with a median of 1 day (IQR 0 to 2 days), compared with cases aged one year or over, with a median of 2 (IQR 1 to 4 days, p-value 0.024. The timeliness of presentation to the health system to the collection of a specimen for laboratory testing did not vary by patient age, or between hospitals. The interval for the time taken for transport of a specimen from the healthcare facility to SSI varied by geographical location, (p-value < 0.001), and was shorter for samples from facilities within the Capital region, where the NRL is located, median of 1.02 days (IQR 0.8 to 2.9) and was longer for samples from regions further from the NRL laboratory, with the longest from North-Jutland, median of 1.93 days (IQR 1.10–3.10). However, overall 98.1% (7,809/7,691) of the specimens arrived at the NRL laboratory within 7 days of sample collection. The interval for the time for specimens to be transferred to a KMA, for primary diagnostics to be carried out and for positive samples to then be forwarded to the NRL also met the surveillance standards, with 80.6% (1,094/1,357) of submitted specimens arriving at the NRL within 7 days of sample collection. For these samples the KMA of origin and relevant dates was known for 644 specimens, and the interval differed significantly between the various KMAs, p-value < 0.001, the shortest interval recorded, had a median of 1 (IQR 0.80 to 3) and the longest had a median of 31.5 days (IQR 0.91–92.64). Five of the KMAs met the surveillance standard, however two did not, with 62.6% (47/75) and 71.4% (5/7) of specimens arriving at the NRL within 7 days. The timeliness of step (f) sample arrival to results of viral genotyping could be calculated for 2013, results for 82.5% (193/234) of specimens were available within 28 days.

The final time interval was described before symptom onset and reporting of clinical symptoms to IDED. There was no difference between patients aged over and under one. There was a decreasing trend detected in the value of the interval overtime (p-value < 0.001). The year with the longest interval for the collection of clinical data was 2011, with a median of 100 days (IQR 60-183), decreasing to a median of 19 (IQR 5-42) in 2013.

Discussion

This study aimed to review the Danish EV surveillance system and determine whether the system fulfils its objective to document the absence of poliovirus

Table 3

Value of examined time intervals in days, enterovirus laboratory surveillance system, Denmark, 2010–2013

Interval description	N	Median days (IQR)
From symptom onset and presentation to the health system (a)	497	1 (0–3)
From presentation to the health system to the collection of a specimen (b)	475	1 (0–2)
From specimen taking to arrival at the reference laboratory for primary diagnostic (c)	7,961	1.4 (0.9–2.5)
From sample arrival to results of the primary EV diagnostic laboratory test (d)	7,961	1.3 (1–3)
From specimen taking to arrival at the reference laboratory for typing analysis (e)	1,378	3 (1–5)
From sample arrival to results of viral typing[a](f)	1,378	17 (11–26)
From symptom onset and reporting of clinical symptoms to the department of epidemiology (g)	770	33 (17–63)

IQR, 25–75% interquartile range

a-g: Time intervals as indicated in figure 2.

[a]Data for 2013 only.

transmission in Denmark and monitor genotypes of EV in circulation.

From 2010 to 2013, the EV laboratory surveillance system performed detailed surveillance of EV in circulation in Denmark; over 20,000 specimens were tested with the primary aim of documenting the absence of poliovirus transmission. No wild-type poliovirus was detected during this period. The laboratory system has the ability to detect the occurrence of a poliovirus infection; ensured by poliovirus proficiency panels from WHO correctly serotyped through this system within a 14-day time period, following WHO guidelines [18]. The surveillance system additionally gathers the necessary information to facilitate an investigation if a case of poliomyelitis occurs. As a secondary objective this surveillance system also monitors the circulating EV genotypes in Denmark and allows to study long-term patterns and epidemiological characteristics of EV transmission and it facilitates the detection of outbreaks through analysing data for individual genotypes. In addition, the data collected by this system enables the identification of molecular targets for the development of more specific diagnostic assays should they be required, for examples in the case of an outbreak as demonstrated by recent investigation on EV outbreaks in Denmark [3,19,20].

The second part of this study aimed to evaluate the EV surveillance system from 2010 to 2013. Firstly, the completeness was determined as a means to whether the data collected was meaningful and included the entire population that should be covered by the surveillance system [9,10]. Secondly, the timeliness was calculated to detect time-delays in the system, and the identification of factors associated with this delay [21].

The initial step of the EV surveillance system, the forwarding of EV-positive specimens for further viral characterisation from KMAs to the NRL, is a passive step in the surveillance system and therefore a major concern with regards to the data completeness of the system [22]. This evaluation documented that the forwarding of EV-positive CSF and faecal specimens to the NRL for viral typing was overall just short of the surveillance standard specified for the completeness of this step. However, some KMAs participate in the sorting of samples and only send samples with sufficient material for further processing at the NRL. The timeliness of this step was however, despite difference between various regions of Denmark, within the surveillance standards for all regions. When the completeness and timeliness values were stratified a number of factors associated with underreporting were identified. The completeness value varied between the KMAs meeting the surveillance standard in some of the laboratories and not in others. Similarly, there were differences in the timeliness values between the various KMAs, ranging from the shortest median of 0.8 days to the longest of 31.5 days. Differences in surveillance performance on a sub-national level, have been reported on previously in AFP surveillance in the United States (US). In the US, regional reporting was also variable and in some instances did not fulfil the surveillance standards, even though the national system on a whole did so [23]. The difference in the distribution of samples from the various KMAs indicated that the surveillance of EV may not be representative of EV circulation in the entire Denmark. The quality of the data obtained in this initial step of the surveillance will determine the representativeness at all subsequent stages [22]. Therefore, improved forwarding of samples and supplementary faecal sampling from patients with too little residual material in the primary sample would allow for a more representative and timely system.

Furthermore, the completeness was significantly higher for patients one year or younger as compared with those older than one year of age, meeting the surveillance standard for those one year or younger only. Often, EV infections in younger patients tend to be associated with a more severe illness and a specific diagnosis considered more urgent; specimens from younger patients tend to be submitted for laboratory testing more frequently [24,25]. This age-based surveillance bias was similarly reported in the US [24].

These results indicate that in order to obtain a more representative overview of EV circulation an increased awareness in the importance of forwarding specimens from older patients is needed by clinicians and primary diagnostic laboratories.

The completeness of the second step of the surveillance system was adequate. All specimens where sufficient quantity remained after primary diagnosis, above 90%, were subject to genotyping. However, since the completeness of the first step of the surveillance system was 79.5%, the percentage of the total number of EV-positive specimens subject to viral typing analysis was closer to 75% and would increase if more specimens were forwarded to the NRL.

The timeliness met the surveillance standard with a median time interval between specimen arrival and a typing result of between two and three weeks. The overall aim of the viral typing is not for the real-time detection of outbreaks but rather to document the absence of poliovirus transmission along with the detection of general trends in the circulation of EV. The system has met its surveillance objective for EV and poliomyelitis, and enabled the detection of recent outbreaks of EV 71 and D-68 [3,16]. However, our results highlighted a high proportion of › 25% of non-typeable EV among EV positive samples sent to the NRL. The proportion of non-typeable isolates is thought to vary by sample type and typing may be more successful for faecal samples. A somewhat high proportion of non-typeable EV specimens reported in studies that include many sample types is not unusual and has been reported previously [26,27]. This study therefore demonstrated that virus genotyping procedures, need to be improved to increase the number of EV where an amplicon can be obtained to increase the typing result-completeness rate.

The final aspect of the surveillance system to be evaluated was the collection of clinical information in relation to neurological symptoms from EV-positive cases, as an indicator of potential poliomyelitis. Our results show that the collection of this information was below the target ≥ 80% over the four-year period. However, the completeness varied by year and was lowest during 2011. An explanation for the low value that year was that there was a shortage of staff at SSI in 2011. This resulted in a backlog in laboratory testing and a delayed reporting to IDED. A decision was taken not to collect detailed clinical information from cases confirmed as EV-positive, where specimen collection had taken place more than 6 months previously, therefore negatively impacting the completeness of data obtained. The system was revised hereafter, and reporting from the NRL to IDED was changed from monthly to weekly in 2012. The completeness subsequently increased and it was above the surveillance standard for 2013. There was however, a long time interval associated with the collection of clinical information (median 2–3 weeks). The current system is paper-based, and requires clinicians to fill in a standardised form and return it by post to SSI. This time interval could be shortened if it would be replaced by an electronic system, as has been demonstrated for numerous other systems [28-30].

This study demonstrated that there were a number of factors that could be addressed to improve the current laboratory EV surveillance system with regards to its primary objective to document the absence of poliovirus transmission. A detailed report on results of this study will be directly communicated to all KMAs, to ensure that all laboratories are aware of the national recommendation to forward positive CSF and faecal samples to the NRL. This report will highlight the fact that specimens should be sent as soon as possible after the primary diagnostic result, and that specimens from patients of all ages should be forwarded for viral characterisation to rule out infections with poliovirus. This fact will also be communicated to clinicians to ensure the importance of collecting faecal samples from EV-positive patients is understood. In addition, a dialogue between the NRL and the KMAs is required to ensure the NRL is fully aware of the selection criteria for sending specimens at all the KMA. A modification of the surveillance system to collect clinical information on EV-positive cases, from a paper-based to an electronic system is additionally suggested as a means to improve the timeliness of the system.

To improve the representativeness of the EV surveillance in Denmark with regards the secondary surveillance objective to track circulating EV genotypes, a decrease is needed in the proportion of isolates where a non-typeable result is reported. A revision of the laboratory work flow is currently ongoing to address this issue and is focussed on redesigning the assay to first differentiate between species A-D and secondly to develop sequencing primers specific for each species.

Conclusion

The current laboratory surveillance system for EV in Denmark is an important tool to document the absence of poliovirus transmission in the country as well as informing public health officials about nationwide alterations in EV trends. The system is currently operating within all but one of the surveillance objectives adopted for this study. Due to the increased risk of an imported poliomyelitis case in Europe, it is recommended to improve certain steps in the EV surveillance system with regards to timeliness and representativeness. Specifically, an increased awareness might result in increased focus and participation of some KMAs, a revision of the EV typing laboratory methodology to decrease the number of non-typeable EV. Finally, the collection of stool and detailed clinical information in case of any EV-positive CSF should be organised in a manner to make it easy for the clinician and the patient to collect and send the material. For example, the use of an immediate electronic prompting system would address these issues and optimise the surveillance system with regards to its objectives.

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This study was approved by the Danish Data Protection Authority as part of a general permission for performing surveillance studies (registration number 2008–54–0472) Permission was obtained from the MiBa Board for the use of national microbiology data on EV diagnostic outcomes for this evaluation.

Conflict of interest

Not relevant

Authors' contributions

Orla Condell: Responsible for the data analysis, drafting of the manuscript and figures and revision of the manuscript.

Sofie Midgley: Advised and contributed to the study design, data analysis plan and results, reviewed drafts of the manuscript critically and contributed to the figures.

Claus Bohn Christiansen, Ming Chen, Xiaohui Chen Nielsen, Svend Ellermann-Eriksen, Mette Mølvadgaard, Kristian Schønning, Silje Vermedal Hoegh: Each is a representative of a clinical microbiological laboratory and part of the collaborative efforts for EV surveillance in Denmark, each revised the manuscript and approved the final version of the paper.

Peter Henrik Andersen: Responsible for the operation of the clinical data component of the surveillance system, revised the manuscript and approved the final version.

Marianne Voldstedlund: Responsible for the operation of the MiBa database, provided advice and guidance on the use of MiBa data, revised the manuscript and approved the final version.

Thea Kølsen Fischer: Head of the Danish National WHO reference laboratory for Poliovirus laboratory at the Department of Microbiological Diagnostics and Virology, engaged in the project design, reviewed the data analysis and drafts of the manuscript critically.

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