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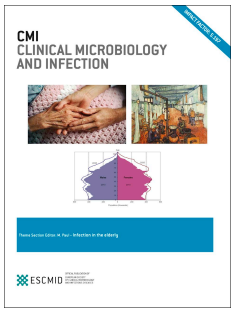

Cryptosporidium infections in Sweden – understanding the regional differences in reported incidence

Dr Heli Harvala, Jessica Ögren, Patrik Boman, Hilde Riedel, Peter Nilsson, Jadwiga Winiecka-Krusnell, Dr Jessica Beser

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Cryptosporidium infections in Sweden – understanding the regional differences in reported incidence

Heli Harvala[1&2], Jessica Ögren[3], Patrik Boman[4], Hilde Riedel[4&5], Peter Nilsson[6], Jadwiga Winiecka-Krusnell[1] & Jessica Beser[1*]

1 Public Health Agency of Sweden, Solna, Sweden, 2 European Programme for Public Health Microbiology Training (EUPHEM), European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden, 3 Microbiology laboratory, Division of Medical Diagnostics, Jönköping, Sweden, 4 Clinical Microbiology, Uppsala University Hospital, Uppsala, Sweden, 5 Department of Medical Sciences, Section of Clinical Bacteriology, Uppsala University, Uppsala, Sweden and 6 Department of Clinical Microbiology, Halland County Hospital, Halmstad, Sweden.

* Corresponding Author for contacts:

Dr Jessica Beser

Public Health Agency of Sweden

Solna, Sweden

E-mail: jessica.beser@folkhalsomyndigheten.se

Corresponding Author for submission:

Dr Heli Harvala

Public Health Agency of Sweden

Solna, Sweden

E-mail: heli.simmonds@hotmail.com

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Sir,

Human cryptosporidiosis is an under-diagnosed gastrointestinal illness caused by protozoa of the genus *Cryptosporidium* [1]. *Cryptosporidium* parasite is detected in stool samples by microscopy with modified acid-fast or fluorescent stains, via antigen detection, or nucleic acid amplification [2]. A total of 404 *Cryptosporidium* infections were notified in Sweden 2014, but the incidence between counties varied from 0 to 34.44 per 100.000 inhabitants.

In order to understand the regional differences in reported incidence of *Cryptosporidium*, an on-line questionnaire regarding the laboratory methods and screening strategies used for *Cryptosporidium* was submitted in October 2015 to 26 clinical microbiological laboratories known to perform parasitological analyses in Sweden. All laboratories responded. Twenty-one laboratories perform *Cryptosporidium* diagnostics, whereas the remaining five laboratories refer their samples for testing elsewhere. Most laboratories (n=13) use the microscopy-based modified Ziehl-Neelsen staining method (mZN) after formol-ethyl acetate concentration, the currently recommended reference method in Sweden. Eight laboratories use a multiplexed PCR including detection of *Cryptosporidium*, and four of them also perform mZN. Five laboratories introduced PCR testing after October 2014. Laboratories use different algorithms for *Cryptosporidium* testing; two laboratories analyse all stool samples from patients with gastrointestinal symptoms regardless of request by PCR, 12 perform *Cryptosporidium* analysis on all stool samples referred with a parasite request (one with PCR; 11 by microscopy on formol-ethyl acetate concentrated wet-smears and then confirm the existence of *Cryptosporidium* oocysts with mZN) and the remaining six laboratories only analyse for *Cryptosporidium* if clinician has specifically requested so (by mZN). Data was missing for one laboratory.

The calculated mean incidence of *Cryptosporidium* was significantly higher in Halland, Jönköping and Uppsala counties in 2014 (34.44 [95% confidence interval {CI} 32.72-36.16]/100.000, 21.40 [95% CI 20.33-22.47]/100.000, and 15.01 [95% CI 14.26-15.76]/100.000, respectively) than in the remaining 18 counties (2.00 [95% CI 1.90-2.10]/100.000; p=0.006 by a non-parametric Kruskal-Wallis test, Figure 1). The same was observed in 2015. These incidences were higher than previously reported from Europe [3]. These counties all analyse a broad range of fecal samples for *Cryptosporidium* either by PCR or microscopy. Uppsala and Jönköping counties analyse all stool samples referred with parasite request for *Cryptosporidium*, whereas Halland county analyses all diarrheal stool samples. In these counties, the positivity rates varied from 1.3% (Uppsala; 62/4941; 95% CI 1.2-1.4) and 1.6% (Jönköping; 115/7140; 95% CI 1.5-1.7) to 2.3% (Halland; 123/5391; 95% CI 2.2-2.4) in 2014. A previous English study identified positivity rate of 0.6% for laboratories testing all stool samples for *Cryptosporidium*, but the method used was not analysed [4]. The incidence was lower in those counties referring the samples elsewhere for testing or analysing samples only upon a specific *Cryptosporidium* request. Concerning is that several laboratories state they screen wet smears for *Cryptosporidium* as a routine procedure, but report very low number of cases. *Cryptosporidium* oocysts are very small and difficult to detect reliably by wet smears without mZN staining; screening wet smears is not recommended for *Cryptosporidium* diagnosis [1,2]. However, the laboratory in Jönköping distinguishes from the other laboratories. Despite using the wet smear screen approach they find a large number of cases, which is likely due to experienced and well-trained staff. The possibility that the variation in incidence between counties is due to other reasons than diagnostic procedures was not addressed here.

Based on the assumption that the incidences obtained in Halland, Uppsala and Jönköping counties 2014-2015 are more accurate than currently reported numbers from counties with lower incidences, a national incidence was calculated. It was much higher than currently reported (22.85 [95% CI: 21.70 - 23.99] versus 5.47 [95% CI: 5.20 – 5.74]). Furthermore, according to our estimations over 2000 *Cryptosporidium* infections would have been diagnosed in Sweden in 2014 (or 2015) if all diarrheal stool samples submitted with parasite request, would have been properly tested for *Cryptosporidium*. This is over four-times more than currently reported. Moreover, these are the estimated figures of diagnosed cases still constituting only a minority of total cases as most patients might not seek healthcare or are not properly sampled.

Molecular techniques including PCR clearly provide an improved workflow enabling more testing; some studies have also demonstrated their improved sensitivity for detection of *Cryptosporidium* compared to mZN [2,5]. Microscopy, on the other hand, has the advantage of being a non-selective method which detects all parasites present in the sample. It is an alternative method reaching comparable sensitivity to PCR but only if combined with appropriate staining and trained laboratory personnel. How the expertise in the field of parasite morphology can be maintained within the high-throughput microbiological laboratories needs to be discussed further. However, analysing a broad range of fecal samples with an adequate method for *Cryptosporidium* is of public health significance. Correct diagnosis is not only important for the patient but also helps to prevent secondary transmissions and outbreaks.

In conclusion, the variation observed in the incidence of *Cryptosporidium* across the Swedish counties reflected both variation in detection methods used and variation in testing algorithms

applied. When interpreting the incidence of *Cryptosporidium* at national and international level, it is important to be aware of the impact of different diagnostic methods and testing algorithms used on the number of diagnosed cases as a potential artefact behind reported regional differences.

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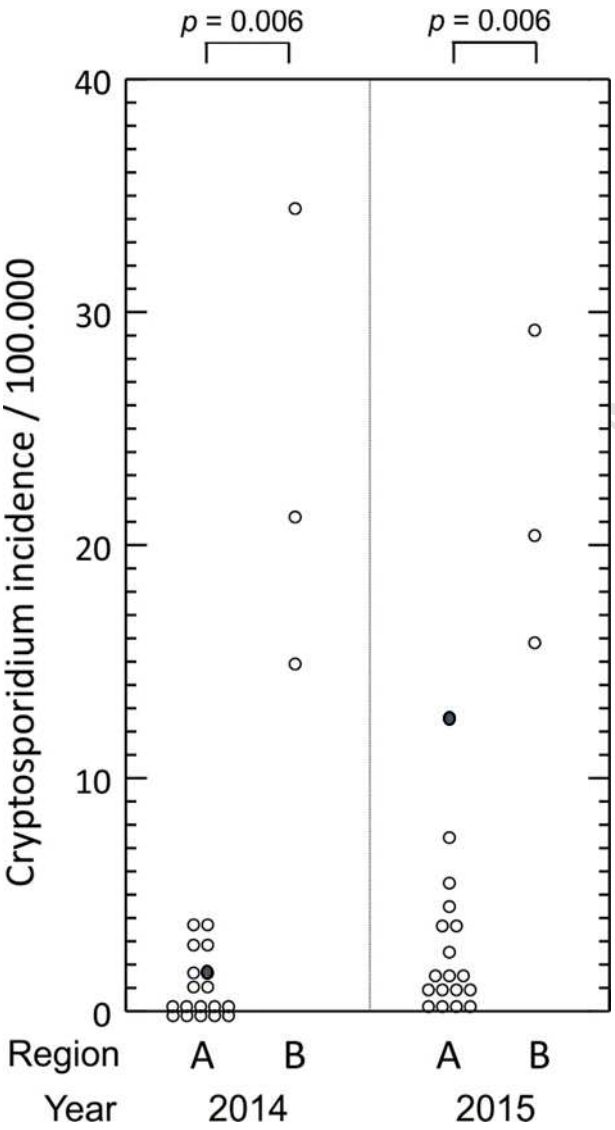
Potential conflict of interest

No reported conflict of interest.

Figure 1. Each dot represents an incidence of *Cryptosporidium* per 100.000 people within a single county. The calculated mean incidence of *Cryptosporidium* in the 18 Swedish counties (Region A) in comparison to the mean incidence in the Uppsala, Halland and Jönköping counties (Region B) in 2014 and 2015. One county has been marked with a grey dot; they introduced PCR-based screening including *Cryptosporidium* of all fecal samples in June 2015.



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