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# SHORT REPORT
# An audit of *Cryptosporidium* and *Giardia* detection in Scottish National Health Service Diagnostic Microbiology Laboratories

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## SUMMARY

*Giardia duodenalis* and *Cryptosporidium* species are protozoan parasites capable of causing gastrointestinal disease in humans and animals through the ingestion of infective faeces. Whereas *Cryptosporidium* species can be acquired locally or through foreign travel, there is the misconception that giardiasis is considered to be largely travel-associated, which results in differences in laboratory testing algorithms. In order to determine the level of variation in testing criteria and detection methods between diagnostic laboratories for both pathogens across Scotland, an audit was performed. Twenty Scottish diagnostic microbiology laboratories were invited to participate with questions on sample acceptance criteria, testing methods, testing rates and future plans for pathogen detection. Reponses were received from 19 of the 20 laboratories representing each of the 14 territorial Health Boards. Detection methods varied between laboratories with the majority performing microscopy, one using a lateral flow immunochromatographic antigen assay, another using a manually washed plate-based enzyme immunoassay (EIA) and one laboratory trialling a plate-based EIA automated with an EIA plate washer. Whereas all laboratories except one screened every stool for *Cryptosporidium* species, an important finding was that significant variation in the testing algorithm for detecting *Giardia* was noted with only four laboratories testing all diagnostic stools. The most common criteria were 'travel history' (11 laboratories) and/or 'when requested' (14 laboratories). Despite only a small proportion of stools being examined in 15 laboratories for *Giardia* (2%–18% of the total number of stools submitted), of interest is the finding that a higher positivity rate was observed for *Giardia* than *Cryptosporidium* in 10 of these 15 laboratories. These findings highlight that the underreporting of *Giardia* in Scotland is likely based on current selection and testing algorithms.

**Key words**: Audit, *Cryptosporidium*, *Giardia*, laboratory testing, Scotland.

## SHORT REPORT

*Cryptosporidium* species and *Giardia duodenalis* (syn. *lamblia* or *intestinalis*) are the most common intestinal protozoan parasites to infect humans in the UK with both pathogens being notifiable under the Public Health (Scotland) Act 2008. Clinical symptoms include diarrhoea, cramps, malabsorption, bloating, pyrexia,

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nausea and vomiting. In addition, *Giardia* can induce excessive flatulence and foul-smelling belching and can result in long-term complications, including IBS-like symptoms [1]. Usually no drug treatment is required for immunocompetent cryptosporidiosis cases, whereas giardiasis is a treatable disease.

*Cryptosporidium* and *Giardia* are acquired through the ingestion of faeces containing infective oocysts or cysts respectively. Outbreaks of cryptosporidiosis have been associated with water sources, including drinking water and recreational water [2]. Petting farms have also been implicated in human disease, as have food sources including pre-cut salads [3, 4]. Likewise, large outbreaks of giardiasis have been associated with contaminated drinking water [5]. Reports also indicate foods as potential sources of *Giardia* as well as companion and farm animals [6–11]. However, further studies on sources and transmission routes are crucial as data to support the importance of animal transmission is lacking, particularly within the UK.

Current recommendations for the laboratory testing of these pathogens in the UK are described in the Standard Microbiological Investigations (SMIs) developed by PHE (Public Health England) in conjunction with the NHS (National Health Service) and additional microbiological societies. There are three SMIs, which are relevant to the investigation of *Giardia* or *Cryptosporidium*, namely B30, B31 and S7 [12–14]. SMI B30 describes pathogens commonly associated with gastrointestinal infections and includes the reporting of *Cryptosporidium* and other parasites [12]. SMI B31 describes the sample types and methods to detect parasites [13], whereas SMI S7 describes algorithms for investigative processes to inform users, which tests to perform based on clinical presentation [14]. *Cryptosporidium* and *Giardia* are both stated in SMI S7 where *Cryptosporidium* is considered as first line testing in patient's presenting with acute diarrhoea and/or vomiting, whereas *Giardia* is considered as part of secondary testing [14]. Whilst *Cryptosporidium* is acquired in the UK and can also be associated with foreign travel, *Giardia* is commonly mis-conceived as being mostly travel-associated with the current SMIs reflecting this. Therefore many laboratories only process a small selection of stools for *Giardia* investigations. This is likely to result in stools from locally acquired cases, and cases where travel history has been omitted from the request form, failing to be tested in Scotland (C.L. Alexander, manuscript in preparation).

As described in SMI B31, the current method for *Cryptosporidium* detection is microscopy where faecal smears are fixed and stained using auramine-phenol (AP), modified Ziehl–Neelsen (mZN) or both. Similarly, microscopy is also used for identification of *Giardia* via direct examination of a wet preparation with or without the addition of iodine. Enzyme immunoassays (EIA) are available for both *Cryptosporidium* and *Giardia*, including combination kits, which can detect both pathogens simultaneously, but they do not distinguish between them. Advances in molecular detection methods permit the use of multiplex panels, which include reagents to detect *Cryptosporidium* and *Giardia* from nucleic acids extracted from stool.

The aim of this audit was to explore testing algorithms and selection criteria across Scotland for the identification of *Cryptosporidium* and *Giardia* in diagnostic microbiology laboratories. This would capture information to determine if variation in detection rates across health boards would be explained by differences in testing algorithms or identification procedures. The audit design was based on a similar audit on English and Welsh data, which examined only *Cryptosporidium*, not *Giardia* [15].

The Scottish audit comprised series of questions on sample acceptance criteria, current and future sample testing methods and testing rates for both pathogens. It was circulated via the SMVN (Scottish Microbiology and Infection Network) in February 2015 to 20 diagnostic laboratories covering all 14 territorial health boards in Scotland reaching Consultant Microbiologists and senior Healthcare Scientists. Results were returned to Health Protection Scotland (HPS), imported to Microsoft Access database and analysed using the SPSS analytical software (version 21) and Microsoft Excel. Data from each laboratory was anonymised by assigning a unique identifier, e.g. ID 1, ID 2, etc. to every laboratory.

Following one reminder email, 95% (19/20) of the questionnaires were returned by June 2015, representing all 14 health boards. Of the 19 laboratories that responded, 18 tested all diagnostic stool samples for *Cryptosporidium* species with one laboratory (ID11) selectively testing samples for *Cryptosporidium* using criteria of travel history or when specifically requested. Sample selection for *Giardia* testing was much more diverse. Four laboratories (ID2, 10, 13, 17) tested for this pathogen in all diagnostic stools.

Laboratories were questioned on the number of stools tested for either pathogen (Fig. 1) and the number of positives identified during the period from 1 April 2014 to 31 March 2015. Laboratories ID 7 and 8 did not provide this information. In total,

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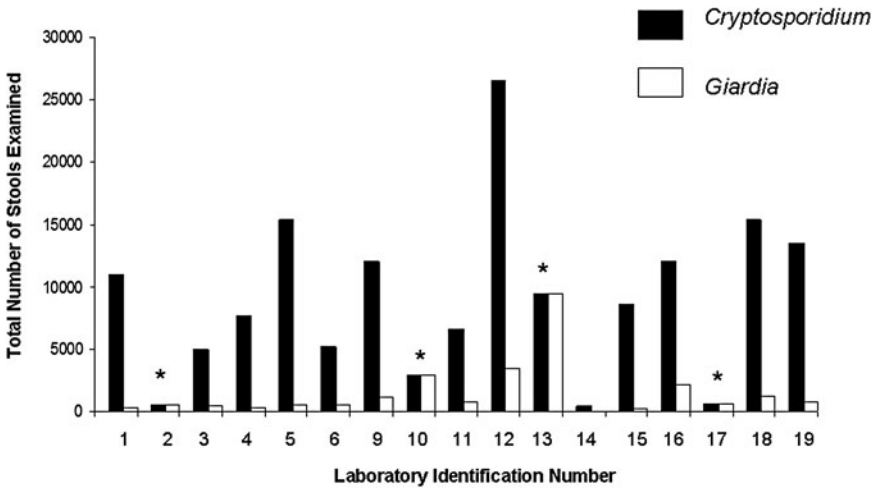


**Fig. 1.** Total number of stools tested for *Cryptosporidium* and *Giardia* between April 2014 and March 2015. Laboratories with identification numbers 7 and 8 did not provide the requested information. *Denotes those laboratories that screened all stools for both *Giardia* and *Crpytosporidium* (Laboratories 2, 17 performed manual EIA. Laboratories 10 and 13 performed microscopy with AP staining to detect *Cryptosporidium* oocysts. To detect *Giardia* cysts, microscopy in the absence of any stain was used by Laboratory 10, whilst Laboratory 13 used microscopy with iodine).

sixfold higher numbers of stools were examined for *Cryptosporidium* compared with *Giardia* ($n = 152\,954$ *vs.* $n = 25\,185$ respectively). The percentage positivity for *Cryptosporidium* ranged from 0% to 0·76% (data not shown) with 400 laboratory confirmed cases reported. Of note was that two laboratories (IDs 6 and 11) did not detect any *Cryptosporidium* despite screening all stools for this pathogen. It has been shown that *Cryptosporidium* displays geographical variation across Scotland and therefore this finding is not unexpected [2]. With the exception of the four laboratories that screened all stools for both pathogens (ID numbers 2, 10, 13, 17), the number of samples tested by each laboratory for *Giardia* was much lower than for *Cryptosporidium* (ranging from 2% to 18% of the total numbers of stools received; Fig. 1). Despite much lower numbers being examined, the positivity rate for *Giardia* was higher than *Cryptosporidium* in 10 of the laboratories (ID 1, 4, 5, 9, 12, 14, 15, 16, 18, 19) where the percentage positivity for *Giardia* ranging from 0% to 3·71% (Fig. 2) with 166 laboratory-confirmed cases. The highest percentage positivity for *Giardia* (3·71%) was achieved by Laboratory 19 with 27 reported cases from 728 stools tested. This laboratory examined stools for *Giardia* by microscopy when a foreign travel history was stated, which occurred in 5% of stools submitted (728/13 473) and no concentration step was performed prior to microscopy examination.

Being mindful that only a small selection of stools were examined for *Giardia* in these laboratories, the results suggest that the absolute numbers of reported *Giardia* cases is likely to be much greater if testing were extended to include all stools. Of course differences in the percentage of positives between laboratories may be attributed to differences in selection criteria and testing methods. In addition, it is likely that *Giardia* exhibits varied geographical distribution similar to *Cryptosporidium* with possible 'hot spots' of pathogen existing locally. This is supported by the finding in those laboratories from different health boards that screened all stools for both pathogens (ID numbers 2, 10, 13, 17), where Laboratories ID 2 and 17 did not identify any *Giardia*-positive samples. However, it should be noted that these findings are based on the testing of very small numbers of stools from Laboratories 2 and 17; therefore the lack of parasites is not unexpected. In contrast, Laboratory 10 reported fewer *Giardia* positives than *Cryptosporidium* positives, whilst Laboratory 13 reported comparable numbers of both pathogens.

Information was also provided on the testing methods as there are now a range of tests in addition to microscopy to detect parasites. These include EIAs, immunofluorescent antibody tests, rapid diagnostic cassettes and molecular-based assays. For *Cryptosporidium*, the majority of laboratories (17/19) performed microscopy to detect oocysts. The most common staining method chosen was AP confirmed by mZN in eight laboratories (ID 1, 3, 5–7, 11, 16, 19). Three laboratories performed mZN alone (ID 9, 14, 18), six employed AP staining only (ID 4, 8, 10, 12, 13, 15), one laboratory used a

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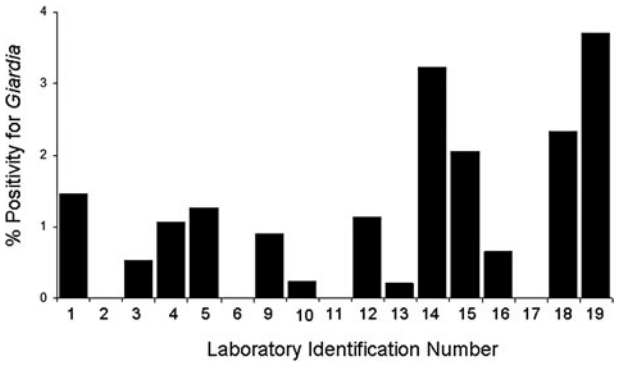


**Fig. 2.** The percentage positivity of *Giardia* in stools. Laboratories with identification numbers 7 and 8 did not provide the information requested.

lateral flow immunochromatographic antigen assay (ID17), and another performed a manually washed plate-based EIA (ID 2). This is a similar to laboratory practices elsewhere in the UK where a survey in England and Wales performed during 2015 to assess *Cryptosporidium* found 54 of 85 (64%) responding laboratories used AP, 14 (16%) mZN, 16 (19%) EIA and one laboratory used PCR [15].

To identify *Giardia* cysts, all laboratories employed bright-field microscopy wet preparations, with the exception of the following: one laboratory referred samples directly to the SPDRL (Scottish Parasite Diagnostic and Reference Laboratory), one performed a lateral flow immunochromatographic antigen assay (ID17), and another used a manually washed plate-based EIA. Of the 16 laboratories using microscopy to identify *Giardia*, half of the laboratories (8/16) concentrated samples first, with about one-third of laboratories (5/16) 'sometimes concentrating samples' for *Giardia* and 3/16 not providing an answer. The use of stains to assist with a *Giardia* identification was variable with half of the laboratories staining with iodine (ID 1, 3, 7, 9, 13, 15, 16, 18), whilst the remainder not using any stain.

The high response rate of users permitted an accurate reflection of the current status of *Cryptosporidium* and *Giardia* testing across all 14 health boards in Scotland. Although the analysis of laboratory practices for *Cryptosporidium* detection appear to be standard across the majority of Scottish laboratories, significant differences were highlighted for both the sample selection criteria and testing techniques for *Giardia*. Algorithms are devised using the SMIs for guidance, which is essential to conform to UKAS (United Kingdom Accreditation Service) standards. Variations arise as laboratories seek the most suitable methods based on sample size, and take into consideration the numbers and experience of staff, sample turnaround and overall laboratory budget. In addition, the SMIs are subject to interpretation, contributing to different algorithms being implemented. The main difference observed in this audit was the large discrepancy between sample selection criteria for *Giardia* testing with 12 laboratories using more than one criteria, the two most common being a 'recent foreign travel' history ($n = 11$) and 'testing upon request' ($n = 14$) (Laboratories 1, 3, 4, 6–9, 10, 11, 14, 15, 18). One laboratory (ID 14) tested for *Giardia* in specific months in addition to 'when requested' or where 'persistent diarrhoea' was stated. One further laboratory (ID 3) tested immunocompromised patients in addition to those with a travel history or when requested. Laboratory ID16 did not provide details on the testing criteria. Clinical information stating 'persistent diarrhoea' was not included in the questionnaire; however, two of the laboratories (ID 14, 15) stated this in the additional comments section. Therefore, it is possible that other criterion, including specific clinical details or sample consistency are used across laboratories, but were not assessed in this survey. The consistency of stools is noted in the SMIs where 'liquid or semi-formed' stools should be analysed. However, formed stools can contain either *Cryptosporidium* oocysts or *Giardia* cysts (C.L. Alexander, personal observation). It is also recommended that the concentration of faeces is performed on samples requesting parasite investigations yet the successful concentration of *Giardia* cysts is dependent on a variety of factors. These include sample consistency, age of sample, cyst integrity, storage conditions and sample volume, which impact on efficient cyst recovery. A previous study from 2002 examining the reporting of *Giardia* in Scottish laboratories highlighted similar results to this most recent survey with 23 of a possible 27 laboratories using varying criteria [16]. Since the publication of this earlier report, a number of diagnostic facilities have been consolidated, reducing the number of microbiology laboratories in Scotland. Despite this, these results suggest that the testing algorithm has not changed considerably in the past 13 years. Although the SMI S7 acknowledges that *Giardia* and *Cryptosporidium* are two main parasites, only *Cryptosporidium* is included in first line screening despite giardiasis being a treatable disease. A caveat is included where 'laboratories may wish to consider adding *Giardia* to the primary testing set based on local risk assessment and operational

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capabilities'. However, many laboratories choose not to implement first line screening for historical reasons and to prevent additional workload and associated costs.

Although the SMI B31 correctly informs users that *Giardia* cysts are present worldwide, testing is not recommended in travellers from geographical regions where the assumed risk of infection is low. However, several of the listed regions are known to be prevalent for giardiasis. These include areas popular with UK travellers such as New Zealand, where the local Ministry of Health describes *Giardia* as 'common', and Western Australia where over 700 cases of giardiasis are reported each year. Similarly, in America, the Northern regions have some of the highest rates of giardiasis [17]. In the UK, there is increasing evidence to highlight as many as 75% of giardiasis cases are acquired within this country [18]. In addition, there are reports of regions across Western Europe where recreational and drinking waters have been shown to harbour *Giardia* cysts [19, 20], creating the potential for human disease. However, as giardiasis notification rates across Europe are extremely variable and robust national surveillance systems are lacking, cases are not formally captured. This can be misleading, giving the impression that these regions are 'low' risk. Therefore a significant number of genuine cases are being mis-diagnosed using an algorithm based largely on travel history.

In terms of future testing in Scottish laboratories, at the time of the survey, one laboratory was in the process of validating a plate-based EIA automated with an EIA plate washer. A further six of the 19 laboratories were considering reviewing or were in the process of reviewing their methodologies; three were considering a plate-based EIA automated with an EIA plate washer, two were considering molecular testing and one undecided but reviewing. Laboratories are moving towards automated methods with improved sensitivity as a result of the loss of microscopy expertise due to retirements. These changes will promote improved, consistent testing across laboratories. An increase in positive samples from 10·1 per 100 000 population in 2002 to 33·6 per 100 000 population in 2006 was seen by Ellam *et al.* [21], who introduced a more sensitive *Cryptosporidium*/*Giardia* EIA for all faecal specimens from patients with community acquired diarrhoea, replacing wet preparation. Similarly, Chalmers *et al.* [22] described improved sensitivity using immunochromatographic lateral flow assay (ICLF) and immunofluorescence microscopy compared with modified ZN. Commercial and in-house molecular assays are also available with improved sensitivity to detect either individual or multiple enteric pathogens from a single stool sample. A study by Stensvold and Nielsen [23] described the detection of *Cryptosporidium* DNA in 16 samples that would have been reported negative if microscopy was used. However, when implementing a new test or altering selection criteria, it is important to also assess any disadvantages. One being that improved sensitivity of assays can lead to the reporting of false positives. There are also issues around the increased workload and cost implications should laboratories include *Giardia* in their screening of all stools. A rise in the number of positives reported and identification of potential outbreaks would have wider implications for follow-up from Health Protection Teams and Public Health. However, bearing in mind giardiasis is a disease that can result in long-term issues if left untreated, it is prudent to support the movement towards testing of all stools for both pathogens across the UK [15, 16, 24]. This will involve reviewing the current SMIs to ensure an accurate diagnosis is made to support the timely administration of appropriate treatment. A recently established National *Giardia* Working Group organised by HPS aims to address important issues around testing with support from clinical, academic and Public Health colleagues. In addition, valuable studies are required to generate supporting evidence to describe local sources and transmission routes of *Giardia* in Scotland. Specific assemblages of *Giardia* have been identified in humans within Scotland, termed A and B [24]. Although these assemblages have been identified from companion animals and livestock both in the UK and worldwide [8, 9, 10, 11], the data are limited to small studies with most animals being infected with assemblages other than A and B. Therefore considerable debate exists over the importance of zoonotic transmission of *Giardia* from animals to humans and vice versa. A recent UK-based study described the presence of Assemblage A in 16/63 cattle samples with a further eight cattle harbouring mixed assemblages containing Assemblage A. In 24 sheep samples examined, four were found to have Assemblage A and one harboured a mixture containing Assemblage A [7]. Another UK study examining *Giardia* highlighted that it was the most common parasite in pet dogs with 380/4526 dogs testing positive. However, no further studies were performed to explore Assemblages [8]. Studies in dogs elsewhere show variable results with one study in Germany stating a predominance of Assemblage A, whilst another German study described dogs infected mostly with

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Assemblage D [9, 10]. Despite limited and sometimes conflicting data, the potential exists for animal–human transmission. However, the need for further molecular-based UK-wide studies is essential to gain in-depth information for a better understanding of transmission routes and epidemiology. A recent UK study describes human–human transmission involving asymptomatic carriers in the North West of England where screening of all household contacts of giardiasis cases was performed, with 41/212 contacts being positive [25]. Currently, laboratory testing is not performed on asymptomatic contacts therefore there are likely to be human reservoirs of *Giardia* that go undetected and contribute to local clusters or outbreaks of giardiasis. It is also likely that imported cases induce local spread via human–human transmission. However, there is no data to support this in Scotland as no nationally-funded *Giardia* investigation service for clinical cases exists. Such a service using molecular tools to type isolates is essential to support the management of outbreak investigations. Further work to optimise and standardise a robust molecular typing scheme with high discriminatory power for *Giardia* outbreak investigations is on-going.

This audit has highlighted the need to examine all stools for *Giardia* and to adopt this approach across the UK. This, combined with a National outbreak service offering a robust-typing scheme will ensure cases of this treatable disease are not missed and outbreaks are effectively managed. There is the need for valuable, in-depth molecular studies across the UK to include animal and human samples, in addition to recreational and drinking waters to greatly improve our knowledge and understanding of this increasingly important pathogen.

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## DECLARATIONS OF INTEREST

None.

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