

# The aetiology of infectious intestinal disease in the community in Malta

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## Abstract

Routine sources of data provide limited information on aetiological agents causing infectious intestinal disease (IID) in the community. A retrospective, age-stratified, cross-sectional, telephone study at community level was performed whereby identified cases were asked to submit stools for analysis.

Of a total of 3504 persons who participated, 99 respondents were suffering from IID. Of these, 37.4% ( $n=37$ ) cases submitted stools for analysis. These samples were analysed for bacteria (*Salmonella*, *Campylobacter*, *Escherichia coli*, *Shigella*), protozoa and viruses (rotavirus, norovirus).

*Salmonella goldcoast* was identified in 2.7% ( $n=1$  of 37 tested) of cases, rotavirus in 10% ( $n=3$  of 30 tested) of cases and norovirus in 20% ( $n=6$  of 30 tested) of cases.

This study describes norovirus being the commonest aetiological cause of IID in the community of Malta, which along with the data from the national surveillance system is of value in planning policies for the control of infectious intestinal disease.

## Keywords

Infectious intestinal disease, aetiological pathogens, community study

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## Introduction

Infectious intestinal disease (IID) is still one of the commonest infectious diseases.<sup>1-2</sup> However, data on the pathogens causing IID is limited mainly to routine laboratory-based surveillance. In Malta, a small island state, the Infectious Disease Prevention and Control Unit within the Health Promotion and Disease Prevention Directorate, is responsible for the surveillance of infectious intestinal disease. This unit receives notifications from general practitioners, hospital physicians and laboratories. The majority of notifications received include cases that required hospitalisation or referral for stool culture analysis. Therefore, it is only a small proportion of the actual cases occurring in the community which are actually tested to identify the aetiological pathogen as is illustrated in Figure 1, which represents the reporting pyramid for IID in Malta.

From the current laboratory based surveillance system, the commonest pathogens identified are *Campylobacter*, *Salmonella*, *E. coli* (generic), and a minority of cases of shigellosis, giardiasis, and cryptosporidiosis. A substantial number of notified cases are defined as unspecified where the aetiological agent has not been identified (Table 1).<sup>3</sup> For the unspecified cases, the pathogen may have not been isolated from the specimens or, it may have been a pathogen that was not tested for, or it is one which has not yet been identified. It may well be that the majority of these cases are of viral origin, since tests for viruses are not routinely carried out in Malta unless specifically requested.

The overall trend in bacterial pathogens over recent years in Malta is that of a statistically significant increase ( $p<0.01$ ) in the incidence of campylobacter cases (Figure 2).<sup>3</sup>

A number of studies have been performed in various countries to identify the common microbiological causes of IID, going beyond the information obtained from routine sources.<sup>4-8</sup> In England, population-based cohorts were recruited from practice lists and followed up whilst a nested, case-control, general practitioner (GP) component was carried out. These identified micro-organisms associated with IID at community and GP level.<sup>4</sup> In the Netherlands, cases of gastroenteritis that had consulted a GP and cases from a community-based study were asked to submit stools for pathogen identification.<sup>5,6</sup> Subsequently, a one-year intensified retrospective study of outbreaks of gastroenteritis was carried out to identify the causative pathogens.<sup>7</sup> Sweden embarked on a one-year

prospective study to identify entero-pathogens in cases of diarrhoea.<sup>8</sup> From these studies, the commonest pathogen identified at community level was norovirus and, at GP level, campylobacter (Table 2).

To date there are no known studies in Malta that have attempted to define the relative contributions of different entero-pathogenic bacteria, protozoa and viruses causing IID in the community. This study was conducted as part of a coordinated series of studies on IID that were initiated in 2003.<sup>9-11</sup> This paper describes the main microbiological findings of this study.

## Methods

A retrospective, age-stratified, cross-sectional, telephone study was carried out in order to define the relative contribution of the various pathogens at community level. A random sample of 3513 persons from the community of Malta was interviewed by

means of a structured questionnaire between April 2004 and December 2005.<sup>10</sup> Participants were asked about symptoms of infectious intestinal disease and were defined as cases or non cases based on the following case definitions:

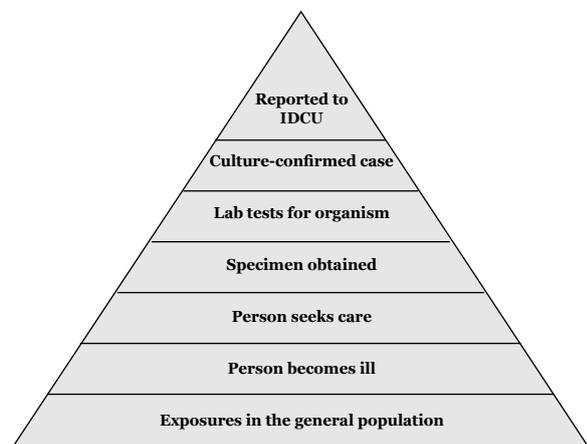
### Case definition for cases

**Inclusion criteria:** individuals who reported at least three episodes of diarrhoea (defined as loose stools) within 24 hours or vomiting at least three times in 24 hours, or suffered diarrhoea or vomiting with two or more additional symptoms in 24 hours, over the previous 28 days. Additional symptoms sought included abdominal cramps, abdominal pain, fever, nausea, blood in stools or mucus in stools.

**Exclusion criteria:** individuals reporting any pre-existing illness or non-infectious conditions diagnosed by a medical doctor in which vomiting/diarrhoea was a symptom, or who were concurrently taking any medications which could cause diarrhoea/vomiting as side effects. This definition was chosen from the case definitions available from literature<sup>5,12-15</sup> and was adapted for the study for Malta.

The persons were interviewed by trained health personnel working in the field of public health and identified cases were asked to submit stool or vomitus, according to the predominant symptom, for analysis in order to attempt identification of the aetiological agent. The appropriate forms and sample bottles were delivered on the same day that the case was interviewed by health personnel. Collection by health personnel was offered if the case was too sick (to do so) or had other problems in delivering the sample to the laboratory. Testing for bacteria, viruses and parasites was performed on the samples. The target pathogens were chosen based on known predominant types from routine surveillance. Samples were analysed at the local Microbiology Department for *Salmonella*, *Campylobacter*, *Shigella*, *E. coli* and rotavirus. Testing for noroviruses was performed at the Istituto Superiore di Sanita` (ISS) in Rome. Stool suspensions

**Figure 1:** Reporting fractions of IID in Malta

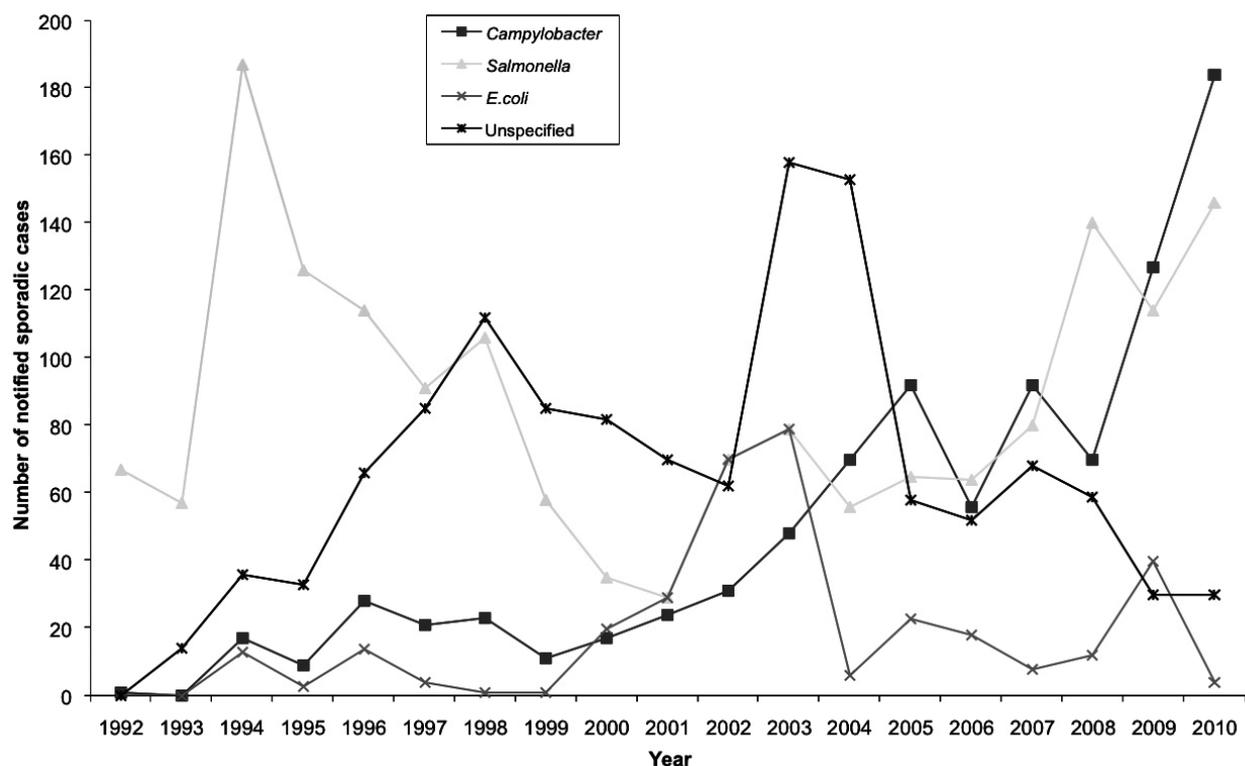


IDCU= Infectious Disease Prevention and Control Unit

**Table 1:** National surveillance of IID in Maltese residents: number of cases reported during 2010 through the national reporting system

Pathogen	No of cases
<b>Sporadic Cases</b>	
<i>Campylobacter</i>	184
<i>E.coli</i>	4
<i>Salmonella</i>	146
Unspecified	30
<b>Cases involved in Outbreaks</b>	
<i>Campylobacter</i>	48
<i>Salmonella</i>	38
Unspecified	69

**Figure 2:** Reported sporadic foodborne illness to national surveillance in Malta, 1992-2010, by aetiological agent.



**Table 2:** Percentage of identified pathogens of IID from international studies

Pathogen	GP studies		Outbreak study	Community study		
	UK GP study <sup>4</sup>	Netherlands GP study <sup>5</sup>	Swedish study <sup>8</sup>	Netherlands outbreak study <sup>7</sup>	UK community study <sup>4</sup>	Netherlands community study <sup>6</sup>
<b>Bacteria</b>						
<i>Campylobacter</i> spp.	12.2	10.4	13.0	1.7	4.2	2.0
<i>Escherichia coli</i> O157	0.1	0.5	8.0	0	0	<1.0
<i>Salmonella</i> spp.	5.0	3.9	7.0	4.0	1.1	4.0
<i>Shigella</i> spp.	0.8	0.1	4.0	0.5	0.1	<1.0
<b>Protozoa</b>						
<i>Cryptosporidium</i>	1.3	2.1	2.0	0.5	0.4	2.0
<i>Giardia</i>	1.0	5.4	2.0	0.5	0.4	5.0
<b>Viruses</b>						
Rotavirus	6.7	5.3	3.0	2.0	4.3	4.0
Norovirus	6.5	5.1	3.0	54.0	7.0	11.0

were prepared in Malta and samples were batched and stored at  $-80^{\circ}\text{C}$  until delivery to ISS.

### Laboratory methods for testing

The fresh stool samples were suspended in saline. This suspension was inoculated on commercial and differential culture media as well as in saline and *Campylobacter* broth. After incubation for 3 days at  $37^{\circ}\text{C}$ , the samples were plated on *Salmonella/Shigella* agar for detection of *Salmonella* and *Shigella* species on days 1, 2 and 3. For detection of *Campylobacter* species the samples were incubated for 3 days at  $42^{\circ}\text{C}$  in a microaerophilic environment with the use of *Campylobacter* medium. For detection of *E. coli* 0157, the samples were incubated for 3 days at  $37^{\circ}\text{C}$  on sorbitol MacConkey agar. These tests were carried out at the local Microbiology Department.

Samples were tested for rotavirus by the use of ELISA at the local Virology Department and for Norovirus by use of reverse transcription-polymerase chain reaction (RT/PCR) at ISS in Rome.

Stools suspensions (10%) were used for viral RNA extraction with the commercial QIAmp<sup>®</sup> Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. This kit provides rapid DNA purification from fresh or frozen human stool or other sample types that have high concentrations of PCR inhibitors. The RNAs obtained were examined by a NoV-specific reverse transcription-polymerase chain reaction (RT-PCR) using the primers JV12-JV13 targeting a 327 bp nucleotide region of the open reading frame (ORF) 1 (*RdRp*). To confirm diagnosis and to characterise strains, a number of NoV positive samples were subjected to nucleotide sequencing of the ORF1 fragment, performed using ABI PRISM BigDye Terminator kit version 2.0 (Applied Biosystems). The sequences corresponding to the ORF1 region were aligned with those present in the FBVE (FoodBorne Viruses in Europe; QLK1-CT-1999-00594) Data Bank and analysed using DNASIS Max software (Hitachisoft).

Samples were analysed for intestinal parasites by means of microscopic examination of fixated samples.

The study was approved by the University of Malta Research Ethics Committee.

## Results

A total of 3513 persons were contacted over a 21-month period from April 2004 to December 2005. Of the persons with whom telephone communication was made at any point in time, 3504 participated, giving a response rate of 99.7%. Of these, a total of 99 respondents met the definition of IID cases resulting in a standardised monthly prevalence of 3.18% [95% CI 0.7%-5.74%] with 0.421 [95% CI 0.092-0.771] episodes of IID per person per year.

The 99 cases were asked by the investigators to submit stool samples for analysis but only 37 (37.4%) submitted stools. No vomitus samples were submitted. Specimens were received between 4 and 33 days after the first onset of symptoms with

a median of 18.76 days. There was no significant difference ( $p=0.92$ ) between the lag-time from onset to submission in the samples that tested positive to those testing negative (20.5:19.9 days). Univariate analysis for association of submission of stool samples with age group, duration of symptoms, health care provider seeking and hospitalisation showed no significance with  $p=0.082, 0.356, 0.102, 0.618$  respectively. Hence it seems that these factors did not seem to affect submission of stools for analysis. Figure 3 shows the percentage of cases who submitted stools and had a positive culture according to the duration of illness. None of the cases who had ongoing symptoms at the time of interview had a positive stool culture.

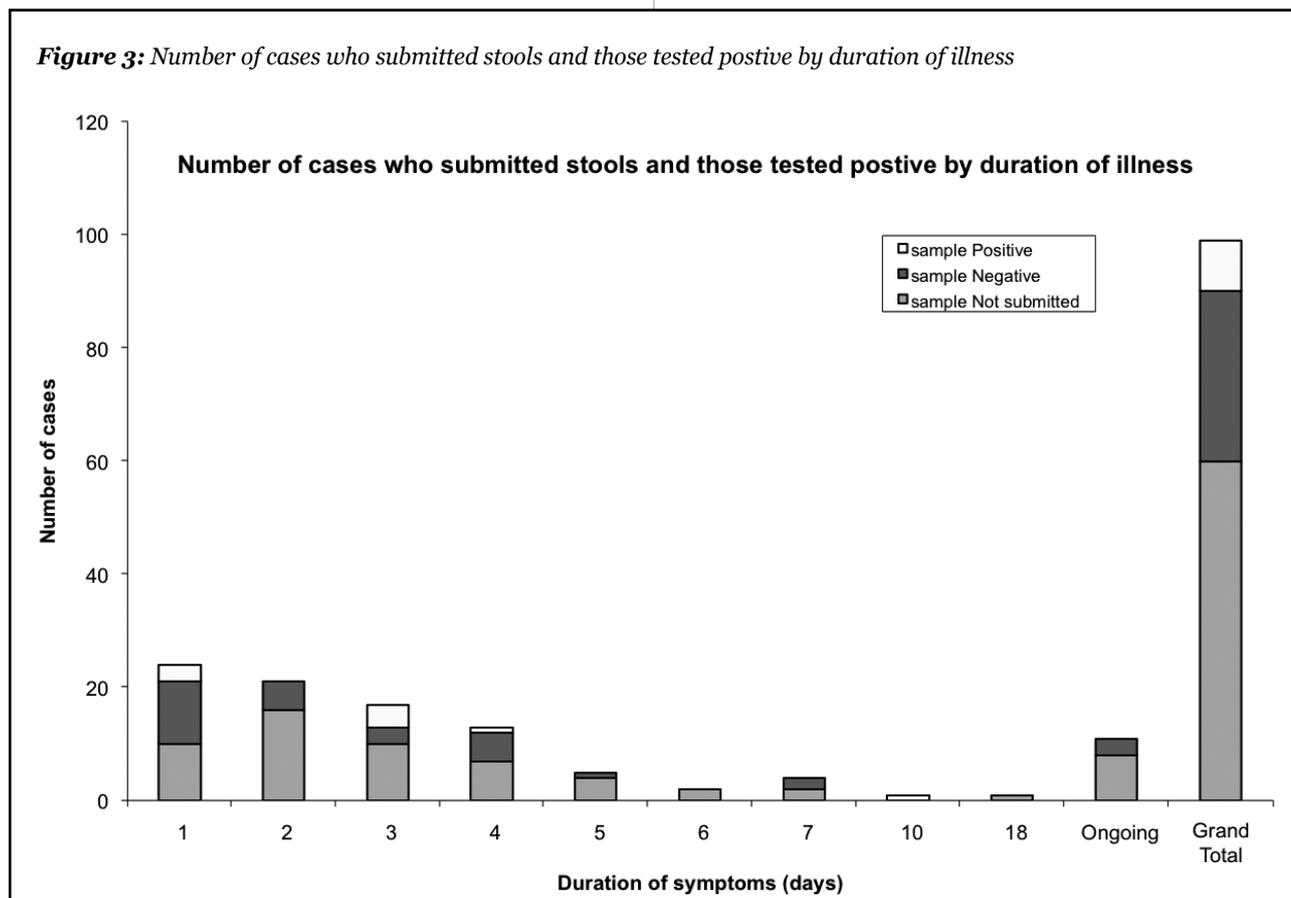
All the samples were sufficient for analysis for bacteria and protozoa ( $n=37$ ) whilst 30 samples were analysed for rotavirus and 30 samples for norovirus. Of those cases who submitted stools, *Salmonella goldcoast* was identified in one case (2.7% of those analysed), rotavirus in three cases (10% of those analysed) and norovirus in six cases (20% of those analysed). Of the six positive cases for norovirus, one was GI.2 (Southampton) and another was GII.4 Lordsdale genotypes (Table 3).

The ages of the cases with positive samples varied by pathogen. A 64-year-old had double pathology with *Salmonella goldcoast* and norovirus. Another case, aged 59 years, had double pathology with rotavirus and norovirus. The other two rotavirus cases were aged 58 and 64 years and the other norovirus cases were aged 1, 10, 39 and 78 years. Figure 4 shows the age distribution of the cases compared to the ones who submitted stools and those having a positive result.

## Discussion

This was the first community-based study to identify the different aetiological pathogens for IID in Malta. Most international studies attempting to identify aetiological agents were prospective cohort studies,<sup>5,12</sup> however these are expensive and time consuming. This study has attempted to recruit participants from a retrospective study in order to identify the aetiological agents implicated. The advantages of such a study are that it is less expensive, less time consuming and does not suffer from loss to follow up which are common problems in cohort studies. However, retrospective studies have other disadvantages in attempting to identify aetiological agents which include non compliance bias and difficulty in isolation of pathogens due to the time lag in submitting specimens. The latter may lead to the identification of persistent organisms and hence may not reflect the overall pattern in aetiology. In international studies identifying pathogens of IID, the predominant pathogen at community level was norovirus with rates of 7 to 11%<sup>4,5</sup> and *Campylobacter* at GP level with rates of 10.4 to 13%.<sup>6,8,12</sup> Similarly, despite the low response rate in submitting samples for analysis in the described study in Malta, the majority of cases were positive for norovirus. Norovirus is one of the human caliciviruses that have a worldwide distribution and has no seasonal incidence except for an apparent predominance in winter. It is the major cause

**Figure 3:** Number of cases who submitted stools and those tested positive by duration of illness



**Table 3:** Aetiological agents identified from symptomatic cases in the IID study in Malta

Aetiological agent	Number tested	Number positive	% positive
<i>Salmonella</i>	37	1	2.70
<i>Campylobacter</i>	37	0	-
<i>E. coli</i>	37	0	-
<i>Shigella</i>	37	0	-
Ova and Parasites	30	0	-
Rotavirus	30	3	10
Norovirus	30	6	20

of epidemic gastroenteritis in both developed and developing countries.<sup>16</sup> The disease is usually mild and self-limiting and occurs mainly in family or community-wide outbreaks, affecting adults, school children, family contacts and young children.<sup>17</sup>

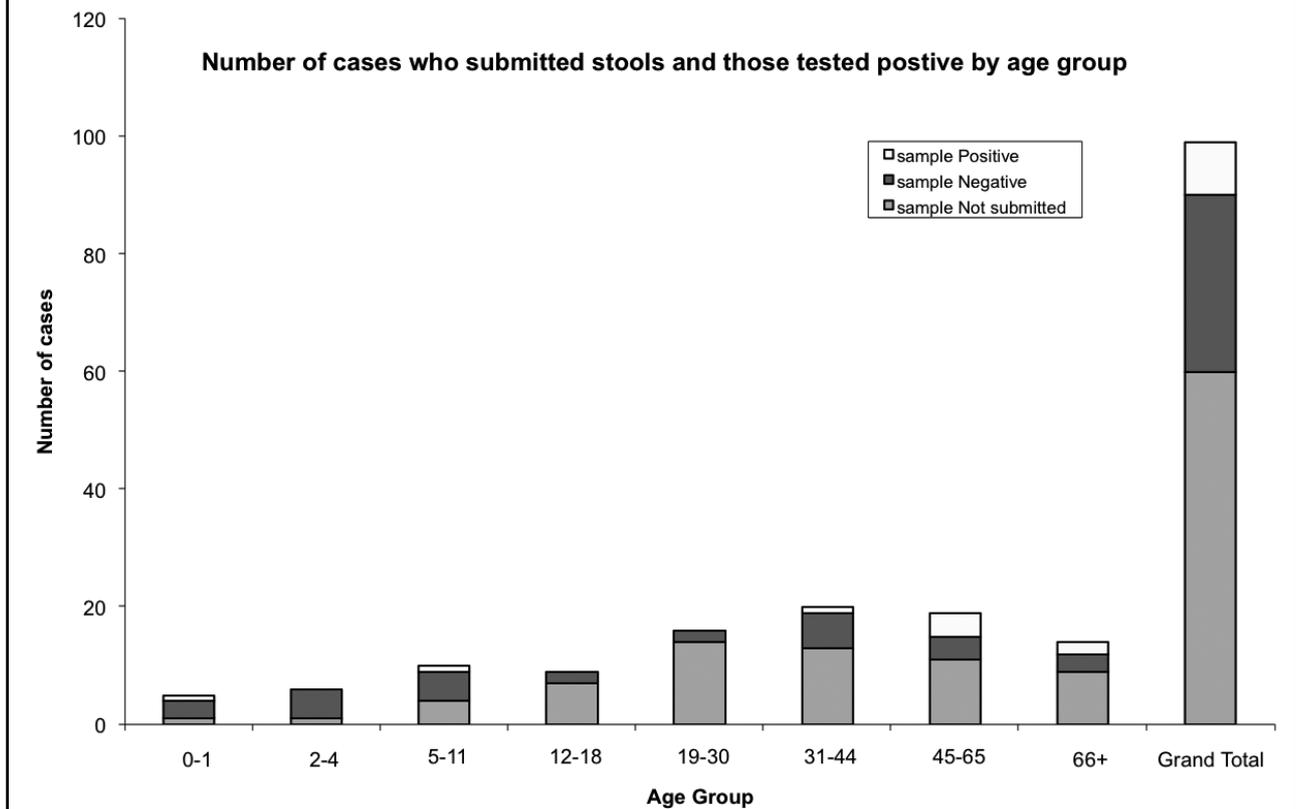
Rotavirus is the target organism most frequently identified in children up to the age of five years in both industrialised and developing countries.<sup>18</sup> A WHO-sponsored review of rotavirus studies found that 20–70% of all hospitalisations and 20% of deaths from diarrhoea in children were attributable to rotavirus.<sup>19</sup> Studies have estimated that 500,000 to 600,000 children die each year because of rotavirus gastroenteritis.<sup>20–25</sup>

The cases identified in the current study were aged more than 50 years. However, the numbers identified were small and not representative.

*Salmonella goldcoast* is only occasionally observed in Malta. From the local national surveillance system, there were no other cases of this serotype in the same year of study. The main *Salmonella* infections seen in Malta are similar to those found in other countries where *Salmonella enteritidis* is the commonest species and *Salmonella typhimurium* is the second largest serotype.<sup>3</sup> The prevalence of *Salmonella* is usually higher in the 0–4 year age group<sup>26</sup> although this was not seen in our study. Similarly other pathogens that are frequently identified from community studies and from national surveillance systems, for example *Campylobacter*, were not isolated.

A large number of samples from the study were negative for the pathogens tested. There are several possible reasons for this. There are some organisms that are known from studies elsewhere to cause IID, but were not tested for in this study. These include *Bacillus spp.*, *Clostridium difficile*, *Clostridium perfringens*, *Listeria*, *Vibrio*, *Yersinia*, adenovirus and others. The absence of pathogens in stool samples that were tested for can be related to the time the sample was taken from the onset of illness. The lag time from onset of symptoms to testing was long and this is known to affect the outcome of the result. Cases could have recovered from their illness at the time of the interview and so the pathogen may no longer have been present in the stools.<sup>27</sup> Prior antibiotic usage could also lead

**Figure 4:** Number of cases who submitted stools and those tested positive by age group



to a negative result. Non-infective causes of intestinal disease can cause symptoms which are very similar to those of IID but the case definition used in the study sought to exclude these cases. Another explanation for negative results could be the low sensitivity of certain methods of detection.

The main limitation in this study was the cooperation rate in the submission of samples. A number of factors could have contributed to this: the patient may have felt better and did not feel the need to comply, the inconvenience of collecting the sample and of delivering it to the laboratory or health centre and the physical inability to collect the sample due to constipation. One major limitation inherent in cross-sectional studies like this is that, for some of the cases, their illness would have resolved by the time of interview and hence there would be lower excretion of pathogens in faeces, which would limit the identification. Another factor is patient compliance since, as mentioned previously, the cases may think that it is not important to perform the test once the illness has resolved. This limitation may be responsible for some of the differences in pathogens identified at various levels of the referral system. Cross sectional studies in other countries including Australia, Canada and Ireland have not attempted to perform analysis on stool samples so no comparison can be made with these. Malta is a small country with a population of about 400,000 persons so one would expect small numbers of persons taking samples for analysis. One option for a higher compliance rate is to perform cohort studies where cases are picked up early in the course of

illness, as conducted in the UK<sup>4,9</sup> and Netherlands<sup>5,6</sup> studies. However, such studies are very expensive and time consuming. Another option is to perform a study on the seroepidemiology of various enteric pathogens.<sup>28</sup> However, the use of such studies in determining disease incidence must be viewed with caution since they do not differentiate between infection and disease. Hence the approach of stool sample analysis was performed, notwithstanding the deficiencies encountered.

Despite the limitations of this study in sample size it has described norovirus as being the commonest aetiological cause of IID in the community of Malta, which along with the data from the national surveillance system would be beneficial in planning policies for the control of infectious intestinal disease.

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