ROBERT KOCH INSTITUT

# EUROPEAN SURVEY ON CAMPYLOBACTER SURVEILLANCE AND DIAGNOSTICS

Campylobacter Infections: Preparing the basis for surveillance in the proposed network for communicable diseases in the European Union

**Final Report** 

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# Abbreviations in alphabetical order

AFLP AMCLI	Amplified fragment length polymorphism Associazione Microbiologi Clinici Italiani
BBSU	Bundesstaatliche Bakteriologisch-Serologische Untersuchungsanstalten
CAP	College of American Pathologists (USA)
CAT	Cefoperazone amphotericin teicoplanin agar
CDSC	Communicable Disease Surveillance Centre
CFU	Colony forming unit
CPA	Clinical Pathology Accreditation
CSM	Charcoal based selective medium
DACH	Deutsche Akkreditierungsstelle Chemie GmbH
DGHM	Deutsche Gesellschaft für Hygiene und Mikrobiologie
EA	European co-operation for accreditation
EARSS	The European Antimicrobial Resistance Surveillance System
EIA	Enzyme Immuno Assay
EQA	External quality assurance
FINAS GBS	Finnish Accreditation Service
mCCDA	Guillain-Barré Syndrome Modified charcoal cefoperazone sodiumdeoxycholate agar
LHA	Local health authority
MIC	Minimum inhibitory concentration
MLA	European multilateral agreements
MLST	Multilocus sequence typing
NPHI	National public health institute
NRL	National reference laboratory
NSC	National surveillance centre
PBP	Peptone broth phosphate
PCR	Polymerase chain reaction
PFGE	Pulsed-field gel electrophoresis
PHL	Public health laboratory
PHLS	Public Health Laboratory Service
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
RIVM	Rijksinstituut voor volksgezondheid en milieu (National Institute of public health and the
	environment)
RKI	Robert Koch-Institut
SIIDC	Swedish Institute for Infectious Disease Control
TSB	Trypticase soy broth
TSI	Triple sugar iron
TTC	Triphenyl tetrasolium chloride
ZLG	Zentralstelle der Länder für Gesundheitsschutz bei Medizinprodukten

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# **Executive summary**

The Robert Koch-Institut in Germany (RKI) has been funded by the European Commission, DG SANCO F/4, to perform a European survey on the surveillance and diagnostics of human *Campylobacter* infections.

The aim of the project was to provide an assessment of the feasibility of a European network for human *Campylobacter* infections. To fulfil the aim, two surveys were conducted. The first survey collected information about laboratory methods in national reference laboratories (NRL) and existing *Campylobacter* surveillance systems. The questionnaire was sent to national public health institutes (NPHI) in 15 Member States and 3 other European countries. The NPHIs participated in the first survey together with the NRLs. The second survey was conducted among 10 Member States and a questionnaire was sent through NPHIs to primary *Campylobacter* diagnosing laboratories to collect information about microbiological methods and diagnostic procedures.

This report summarises the results from both surveys and discussions in the meetings during the project. It also discusses the recommendations and options for a European network for human *Campylobacter* infections.

All countries (n = 18) responded to the first survey. The results were obtained from 15 Member States, Norway, Iceland and Switzerland. All countries except Portugal had a national surveillance system for human *Campylobacter* infections. In 9 countries (Denmark, Finland, Germany, Greece, Iceland, Luxembourg, Norway, Sweden and Switzerland), *Campylobacter* infection was statutory notifiable. Seven countries (Belgium, France, Ireland, Italy, the Netherlands, Spain and the United Kingdom) had sentinel systems which covered part of the country. Austria had both statutory and sentinel systems. In most countries, either laboratories and/or physicians were the notifying partners. In Luxembourg, the physicians were the only notifying partners. In Belgium, Denmark, Finland, France, Greece, Italy, the Netherlands, Spain and Switzerland, the laboratories were the only notifying partners. NPHIs collected information in 14 countries and NRLs in 3 countries. At the European level, the main information flow was thus from the laboratories and physicians to the NPHIs. In 14 countries (82%), the information at European level.

Ten out of 17 countries had developed case definitions for *Campylobacter* surveillance. In Denmark, Finland, Norway, Spain, Sweden, Switzerland and the United Kingdom, the case definition was based on laboratory confirmed diagnoses. Denmark and Finland had defined a time-period for a case. Many countries didn't have a formal case definition but had defined

certain criteria for information to be reported. Belgium, France, Iceland, Ireland and the Netherlands had no case definition for *Campylobacter* surveillance at the time of the survey. However, the cases are all laboratory confirmed in countries with a sentinel system of laboratories. Overall, the case definitions varied from no criteria to carefully defined case definitions between countries. Almost all countries with surveillance over several years showed a steady increase in *Campylobacter* incidences from 1995 to 1999.

From 1995 to 1999, 11 countries reported 154 outbreaks. The highest number (39) of reported outbreaks was in 1997. However, the reporting of outbreaks varied substantially between countries and may not reflect the real situation.

Laboratories performing reference tasks existed in 13 European countries. Eleven of these had a NRL appointed for Campylobacter infections. Two countries had other laboratories with reference tasks. In this report, they are all referred to as NRLs. In 12 countries, the NRLs confirmed the results of primary laboratories. Other common tasks of the NRLs were developing new typing methods (10), developing proposals for standardisation of methods (9), developing new analytical methods (9), and conducting training courses (7). Austria, Iceland, France, Luxembourg and the UK received all isolates from (sentinel) laboratories within a fixed time interval. In Austria, Belgium, France, Germany, Switzerland and the United Kingdom, laboratories sent isolates to the NRL when there was a suspicion of an outbreak occurring . At the European level, only one in every 5 primary laboratories, however, receive the information whether the sample is connected to an outbreak.. Only 13 % of European primary laboratories reported sending tisolated strains to the NRLs for further characterisation. This indicates that strains are not collected centrally by the NRLs. There exists currently no commonly applicable method to subtype Campylobacter strains. Serotyping with commercial antisera is simple, but not all strains are typable with commercial antisera. Therefore laboratories may have to produce the antisera themselves which is very expensive and not possible for all laboratories. Denmark, Greece, Germany and the United Kingdom used serotyping as a sub-typing method. The genotyping methods that were used in the NRLs varied both between laboratories and countries. Phage-typing was in use in UK only. Nevertheless many laboratories, as a consequence of involvement of CAMPYNET, an EU-funded network, now have the facilities and expertise to undertake at least one recommended genotyping method.

The microbiological isolation methods both in the NRLs and primary laboratories were principally the same. The sample was cultured directly on a selective medium/media and incubated at 37°C and/or 42°C in a microaerobic atmosphere. Almost all NRLs (12/13) and most primary laboratories (94%) confirmed the isolates using at least one confirmatory test. The NRL in Sweden performed only genotyping. Some NRLs had given recommendations for

*Campylobacter* isolation. At the European level, one in four laboratories (24%) did not know whether their method was based on published guidelines or not. This indicates a lack of communication between the NRLs and primary laboratories. The mean of positive isolations / 100 investigations (mean isolation rate) ranged from 2.2 to 6.2 by country indicating that there existed differences in investigation practices and/or isolation methods.

Internal quality assurance includes procedures by which a laboratory controls the different steps during the analyses. External quality assurance (EQA) means procedures that are organised by an independent agency which provides controlled material for quality control testing. The external quality assurance offers good opportunities to control the sensitivity and specificity of the whole method. The NRLs in Denmark, Germany and Iceland, had both internal and external guality assurance procedures. Switzerland and the United Kingdom had internal guality assurance and Norway external quality assurance procedures. Six NRLs had not defined any quality assurance procedures. Of internal quality assurance, only quality control of agar plates was requested from primary laboratories. At the European level, about half of the primary laboratories (47%) controlled the quality of agar plates. This was mainly due to the fact that approximately every fourth laboratory (23%) prepared the selective agar plates themselves. All other laboratories bought the plates relying on the control procedures of the manufacturer. Primary laboratories that prepared the plates themselves also controlled the quality of the plates (Spearman  $r_s = 0.88$ , p < 0.01). At the European level, almost half of the primary laboratories (44%) participated in EQA schemes for Campylobacter culturing. In Denmark, England & Wales, Finland and Scotland, all responding laboratories participated in EQA schemes. One reason for the lower participation among other countries was the lack of information. Many laboratories in France (58%), Germany (50%), and Italy (42%) did not know about the availability of EQA schemes in their country. Primary laboratories clearly need stronger support from NRLs in some countries.

Accreditation was not common among the laboratories. NRLs in Germany and Switzerland had accreditation for *Campylobacter* culture according to standards EN 45 001/ISO Guide 25 or ISO 17025. Accreditation was in progress in the NRL in Denmark. The NRL in the United Kingdom had a Clinical Pathology Accreditation which is not test specific. Very few (6%) primary laboratories had accreditation for *Campylobacter* culture.

As the NRLs did not receive many strains for further characterisation, the role of the primary laboratories in antimicrobial susceptibility testing is important. Almost half of the primary laboratories (46%) always performed susceptibility testing for *Campylobacter* isolates. The agents most commonly tested for were erythromycin (92%) and ciprofloxacin (83%). This

provides a good basis for antimicrobial resistance surveillance at the European level. Only in Portugal, was susceptibility testing rarely performed (1/13).

The reported cases of campylobacteriosis are increasing in many European countries revealing that these infections are still emerging. The infection is also included in the list of diseases of a European surveillance system which emphasises the need for EU-wide Campylobacter surveillance. The isolation methods and procedures, as well as the isolation rates, vary between laboratories and countries which raises the issue of standard operating procedures. Sub-typing methods that would be suitable for epidemiological purposes are still under development but most laboratories are prepared to adopt such a sub-typing method when it becomes available. Antibiotic resistance is an important part of *Campylobacter* surveillance and the basis for this exists in Europe. Based on these findings, there is a need and basic infrastructure for a European-wide Campylobacter surveillance network. The main objectives of such a surveillance network would be to provide comparative data on trends between countries and to recognise EU-wide outbreaks among large numbers of apparently sporadic cases. If there is a change, Member States should be encouraged to record, evaluate and communicate the consequences of the change to the system. For sentinel surveillance systems, countries should be encouraged to assess the coverage to achieve comparability in incidence calculations. Countries should be encouraged to include travel information in their surveillance systems. Surveillance systems should be implemented so that trends and effects of intervention measures can be monitored.

The information flow from national I to local level and vice versa, could be improved in many countries. It is important to have at least one laboratory (NRL or other) in each country to support local laboratories and develop method standardisation, which in turn improves case detection. Since one common standardised molecular sub-typing method cannot currently be recommended for epidemiological use on a large scale, it would be advantageous to utilise the expertise of the EU-funded working group, CAMPYNET, within the surveillance network plans. This would involve the appropriate use of existing recommended molecular typing methods for epidemiological purposes.

The European surveillance on human campylobacteriosis could be initiated with the information now available and with a stepwise development over time. To achieve this, a common EU-wide surveillance system should be developed with a common case definition and a minimum data set (age, sex, travel, laboratory information), which would be modified over time. To gain the maximum benefit of such a surveillance network, the information about outbreak investigations should be collected centrally.

### 1. Introduction

In the late 1970's, the development in *Campylobacter* isolation techniques resulted in the discovery of the importance of *Campylobacter* infections. Since then, the thermotolerant *Campylobacter* species (*Campylobacter jejuni* and *Campylobacter coli*) have become one of the most important causative agents of acute bacterial diarrhoea in the industrialised world (1). During the last few years, the annually reported numbers of cases have exceeded the number of Salmonella infections in many developed countries (2). Over 2 million cases are estimated to occur annually in countries like the United States, the United Kingdom or other nations where *Campylobacter* surveillance is established (3).

The course of *Campylobacter* infection varies from asymptomatic carriage to diarrhoea lasting for several days to more than 1 week (3). Fever and abdominal cramps are usually accompanying symptoms. Extraintestinal infections such as bacteraemia and meningitis also occur. The chronic sequelae include reactive arthritis, Reiter syndrome, and an acute paralytic disease of the peripheral nervous system, Guillain-Barré syndrome (GBS). In the United States, it is estimated that approximately 1% of patients with campylobacteriosis develop reactive arthritis 7-10 days after onset of diarrhoea (4). Pain and incapacitation can last for months or become chronic. An estimated one case of GBS occurs for every 1000 cases of *C. jejuni* infection (5). Approximately 20% of patients with GBS are left with some disability and about 5% die despite of advanced respiratory care (4). There is evidence that some serotypes, e.g. O41 and O19, are associated with the development of GBS (5). Increasing incidence and the severity of sequelae indicate that campylobacteriosis has become a major public health problem in Europe.

*Campylobacter* has been isolated from a wide range of domestic and wild birds and mammals as well as from humans. Eighteen species and subspecies have been described, but two (*Campylobacter jejuni and C.coli*) are most frequently associated with human enteric infection (3). The infective dose of *Campylobacter* appears to vary depending on the strain or species and on host factors (6). One study showed that 500 colony forming units (cfu) consumed in milk were sufficient to cause illness (6). The incidence has usually a bimodal age distribution, with the highest incidence occurring in infants and young children, followed by a second increase in young adults 20 to 40 years of age (3).

Infection with enteric *Campylobacter* is seasonal in most countries (7) reaching a peak in the summer and early fall, with the majority of infections apparently being sporadic (3). Outbreaks usually occur in the spring and fall (3). In 1998, a *Campylobacter* food-borne (raw milk) outbreak in Germany affected 186 cases in 6 kindergartens (9) and a water-borne outbreak in Finland

involved more than 2000 people (M. Kuusi, personal communication). Such events demonstrate the potential severity and extent of *Campylobacter* outbreaks.

Prevention is all the more important since antibiotic treatment should be reserved for complicated, severe or invasive infections and is not recommended to be used routinely. Erythromycin has been the most commonly used agent for treating *Campylobacter* enteritis (10). In one study, the effect of erythromycin treatment on the duration of diarrhoea was not clinically significant even when used in an early phase of infection (10). In the 1980s, the introduction of fluoroquinolones offered a new approach to antibiotic intervention. However, the resistance in *Campylobacter spp*. to fluoroquinolones has clearly increased over the past decade in many parts of the world (10). Even resistance to erythromycin has been noted in some countries. Increased rates of resistance raise the need for continuous monitoring of resistance patterns.

A WHO report from 1994 states that the role of typing in *Campylobacter* epidemiology is not yet defined and *Campylobacter* diagnostics and laboratory methods are still developing in the different national reference laboratories throughout Europe (12). The diagnosis also depends on the awareness of physicians to look for *Campylobacter* and reporting is generally lower for diseases mostly treated in outpatients. The detection of *Campylobacter* strains also requires a high level of laboratory expertise which is financially costly and time consuming.

Currently, a European network of communicable diseases is being developed in Europe (http://iride.cineca.org). There is no information about the tools and algorithms used to diagnose *Campylobacter* on the local or national levels in European countries. The aim of this project was to provide an assessment of the feasibility of a European network on *Campylobacter* by collecting information on laboratory methods used for identification of *Campylobacter* as well as on existing surveillance systems. It was anticipated that options for a European Network for Human Campylobacter Infections would be developed on the basis of the accrued information.

The project has been implemented by the Robert Koch-Institut (RKI). The European Commission, DG SANCO F/4, has supported the project. RKI has ensured constant feedback with CAMPYNET, the network on the standardisation of molecular typing methods for *Campylobacter*, with Enter-net and with the European Community Reference Laboratory for the Epidemiology of Zoonoses.

A panel of experts met twice to evaluate the results and define the need and scope of a European network on human *Campylobacter* infections. National institutes of public health in

EU Member States, Iceland, Switzerland and Norway collaborated in the project. The final meeting for all collaboration partners was held in Berlin.

#### 1.1 Methods

To collect information from countries, two mail surveys were conducted:

Survey I: A survey on National Reference Laboratories (NRLs) and Surveillance Centres was performed to describe the surveillance for human Campylobacter infections in 18 European countries, paying particular attention to the general methods and case definitions and to collect information about the tasks of National Reference Laboratories for human campylobacteriosis. The co-operation partners were identified among the NRLs and Surveillance Centres from the 15 Member States, Norway, Iceland and Switzerland.

The questionnaire was prepared by Olav Robstad and Andrea Ammon. Epidemiologists and microbiologists in National Public Health Institutes and national reference laboratories were consulted for the type and formulation of questions. The questionnaire consisted of the following items:

- Existence of National Reference Laboratories (NRLs), tasks of NRLs, methods for *Campylobacter* isolation, identification and molecular sub-typing, quality assurance and accreditation

- Surveillance systems (statutory/sentinel), case definitions, other data sources, reported numbers of cases, recorded outbreaks

*Survey II: A survey on primary laboratories performing Campylobacter diagnostic* was conducted to determine laboratory methods and reporting routines. All countries that participated in the first survey were asked about their willingness to participate in the second survey. Finally, all countries that were willing to participate (10) were included in the second survey.

The questionnaire was prepared by Johanna Takkinen and Andrea Ammon. It was divided into three parts:

*A: Basic information* (laboratory-type, patient groups, total number of investigations, sample transport, routines for testing, submitted patient information)

*B. Sample handling and culture practices* (published method, time interval between sampling and cultivation, procedures before cultivation, cultivation practices, plate

preparation and quality control, confirmation and typing methods, storing and sending isolates, reporting, antimicrobial susceptibility testing)

#### C. External quality assurance (participation, accreditation)

The questionnaire was evaluated in the first expert panel meeting in June 2000 and then sent to five countries (France, Germany, UK, The Netherlands, Ireland) for pretesting. A total of 14 laboratories pretested the questionnaire before final revision.

Each country sampled the laboratories depending on the information available to surveillance centres and the total number of laboratories. In Denmark and Finland, all Campylobacter laboratories in the country were known beforehand and the questionnaire was sent to all of them. In Greece, the questionnaire was sent to public hospital laboratories. In England & Wales, the sampling frame was the laboratories taking part in the national sentinel surveillance for Campylobacter. In Austria, the sampling was targeted to all local health laboratories performing stool diagnostics. In Italy, 192 local health laboratories out of approximately 500 were randomly chosen. In Ireland, the questionnaire was sent to clinical laboratories. In France, 500 laboratories (400 private and 100 hospital) out of about 6000 were randomly chosen. In Scotland, the questionnaire was sent to all laboratories that had reported Campylobacter findings in the previous year. In Portugal, the hospital and private laboratories were randomly chosen respecting the ratio between hospital and private laboratories. So far there has been no centred register or address list about local laboratories in Germany. As there are many private laboratories in Germany, an address list provided by a commercial company was used.

The questionnaire was translated into French (Institut de Veille Sanitaire), German (Robert Koch-Institut) and Italian (Istituto Superiore di Sanità). The data entry of the French questionnaires was performed in France and the data was added to the total database in Berlin. EPI-INFO 6.04 and SPSS 10.07 were used for data analyses.

#### 1.2 Expert panel

The expert panel consisted of five persons, including a representative from Enter-net to build on current knowledge in an existing network, a representative from CAMPYNET to provide recommendations on useful sub-typing molecular tools for surveillance network purposes, and three representatives from national public health institutes (NPHI) in France, Italy and Sweden.

The expert panel consisted of the following group:

- Y. ANDERSSON, epidemiologist, Institute for Infectious Disease Control, SWEDEN;
- A. GALLAY, epidemiologist, Institut de Veille Sanitaire, FRANCE;
- I. LUZZI, epidemiologist, Istituto Superiore di Sanitá, ITALY;
- I. FISHER, Enter-net co-ordinator, Enter-net Surveillance Hub, CDSC, UK;
- J. WAGENAAR, veterinary microbiologist, Institute for Animal Science and Health, the NETHERLANDS (CAMPYNET);
- A. KÄSBOHRER, veterinary microbiologist, Community Reference Laboratory for the Epidemiology of Zoonoses, Federal Institute for Health Protection of Consumers and Veterinary Medicine, GERMANY

The expert panel met twice in Berlin. In the first expert panel meeting, the results from the first survey on national reference laboratories and surveillance centres were presented and the questionnaire for the second survey on primary *Campylobacter* laboratories was evaluated. The second expert panel meeting concentrated on the results from both surveys and developed the preliminary conclusions and recommendations for the final meeting.

#### 1.3 Timetable

1. November 1999	The start of the project
5. May 2000	The deadline for the replies of the first survey (I)
2223.6.2000	The first expert panel meeting
22.12.2000	The deadline for the replies of the second survey (II)
15.1.2001	The second expert panel meeting
16.1.2001	The final meeting for all collaboration partners
August 2001	Draft report sent to collaboration partners
March 2002-03-07	Final report

### 1.4 Final meeting

The final meeting for collaboration partners was held in Berlin on 16<sup>th</sup> January 2001. All members of the expert panel and a representative from each collaborating institute were invited. The participants of the final meeting were:

- G. Feierl, Institut of Hygiene, Austria;
- A. Vellinga, Scientific Institute of Public Health-Louis Pasteur, Belgium
- K. Olsen, Statens Seruminstitut, Denmark;
- P. Ruutu, National Public Health Institute, Finland;
- A. Gallay, Institut de Veille Sanitaire, France;
- A. Ammon, Robert Koch-Institut, Germany;
- T. Breuer, Robert Koch-Institut, Germany;
- J. Takkinen, Robert Koch-Institut, Germany;
- T. Kuczius, Hygieneinstitut, Hamburg, Germany;
- S. Chatzipanagiotou, National and Kapodistrian University of Athens, Greece;
- D. Whyte, National Disease Surveillance Centre, Ireland;
- M.L. Monteiro, Instituto Nacional de Saúde, Portugal;
- J. Cowden, The Scottish Centre for Infection and Environmental Health, Scotland;
- **A. Käsbohrer**, Community Reference Laboratory for Zoonoses, Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin, Germany;
- F. Schneider, Laboratoire National de Santé, Luxembourg;
- Y. Van Duynhoven, National Institute of Public Health and Environment, Netherlands;
- J. Wagenaar, Institute for Animal Science and Health, Netherlands;
- V. Hasseltvedt, National Institute of Public Health, Norway;
- G. Pezzi, Instituto de Salud Carlos III, Spain;
- Y. Andersson, Institute for Infectious Disease Control, Sweden;
- I. Fisher, Enter-net Surveillance Hub. Communicable Disease Surveillance Centre, UK;

The final meeting discussed about the results of the two surveys and preliminary

recommendations and proposals prepared by the expert panel.

#### 1.5 Responses of the two surveys

All countries (n = 18) responded to the first survey for NRLs and surveillance centres (table 1). The second survey was conducted in 10 EU Member States through collaboration partners in National Public Health Institutes, Surveillance Centres and National Reference Laboratories. A total of 2487 questionnaires was sent to primary laboratories and 1014 (41%) replied. Of 1014 laboratories, 695 (69%) performed stool diagnostics and 622 (61%) also *Campylobacter* diagnostics. One laboratory reported using ELISA test for stool samples and was therefore not included in the analyses. The response rates varied from 17% in England & Wales to 93% in Ireland.

#### **1.6 Acknowledgements**

The project group at RKI thanks all the collaboration partners and institutes for their patience and valuable co-operation. Our special thanks go to the expert group for good support during the project.

#### 2. Results of the first Survey (Survey I)

#### 2.1 Campylobacter surveillance systems

Seventeen countries out of 18 have surveillance systems (figure 1). Portugal has no surveillance system so far. In 10 countries, Austria, Denmark, Finland, Germany, Greece, Iceland, Luxembourg, Norway, Sweden, and Switzerland, *Campylobacter* infection is statutorily notifiable. Austria, Denmark, Finland, Germany, Greece, Norway, Sweden, and Switzerland have statutory surveillance systems which have been established since 1996 or earlier. In Iceland and Luxembourg, the statutory notification has been established since 1999 and 2000 respectively (table 2).

Eight countries, Austria, Belgium, France, Ireland, Italy, the Netherlands, Spain and the United Kingdom, have no statutory notification for *Campylobacter* infections but have sentinel systems (figure 1). Austria, Belgium, France, Ireland, the Netherlands and Spain have sentinel surveillance systems for *Campylobacter* since 1996 or earlier (Austria has both a statutory and a sentinel system). In Italy and the United Kingdom, a sentinel surveillance system has been established since the beginning of 2000 (table 2).

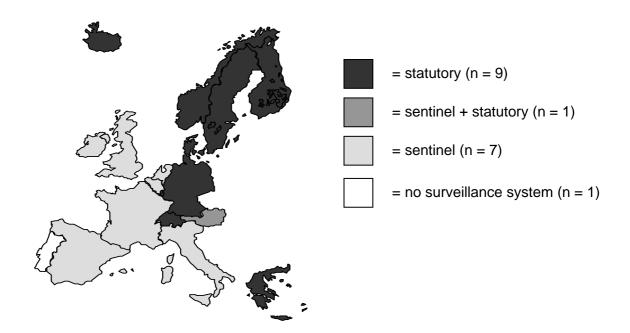


Figure 1. *Campylobacter* surveillance systems in European countries (n=18), Survey I, 2000.

Ad hoc or other sources of data on *Campylobacter* exist in 5 countries. In 1999, Ireland had a nation-wide health board survey of laboratory diagnosed cases of *Campylobacter* to obtain incidence data for 1999 and to look for regional variation in observed rates. The zoonosis section of the Irish Department of Agriculture also compiles statistics on *Campylobacter* in humans annually. In Italy, an additional source to collect data about *Campylobacter* infections is through a system where laboratories that isolate *Campylobacter* spp. report their findings using the same questionnaire as the Italian National Public Health Institute (Istituto Superiore di Sanità) for collecting data on infectious enteritis. The Netherlands collected data in two epidemiological studies: they carried out a sentinel study in general practices (case-control study and enumeration) from 1996–1999 and a population-based cohort study from December 1998 through December 1999. Portugal reported ad hoc sources from sporadic studies as their only source of data on human campylobacteriosis. In addition to the newly established enhanced epidemiological surveillance, the United Kingdom collects information through laboratory confirmed reports of both sporadic and outbreak cases of *Campylobacter* to the PHLS-CDSC.

#### 2.1.1 Co-ordination of surveillance and information flow

In countries with statutory surveillance, 2 require all physicians, who diagnose a *Campylobacter* infection, to notify, two require all laboratories, two countries require both physicians and laboratories and two countries require local health authorities in addition to physicians and laboratories to notify the diagnosis of *Campylobacter* (table 3). National public health institutes (NPHI) collected the surveillance data in 9 countries and the NRL collected the data in one country.

In all 8 countries with sentinel systems, the notifying partners were laboratories, in addition in 2 countries hospitals and in 2 countries local health authorities were also notifying partners (table 3). Also in countries with sentinel systems, the NPHIs collected the data in 5 countries and the NRLs in 2 countries. In Ireland, there existed two separate regional laboratory sentinel systems (LSS and INFOSCAN) which had no systematic collation. Both systems were population based and all laboratories in the regions contributed. Austria had a statutory system for physicians and a sentinel system for laboratories with the physicians notifying to the NPHI and sentinel laboratories notifying to the NRL. In France, the sentinel laboratories were hospital-based and they notified to the NRL.

Altogether, NPHIs collected *Campylobacter* surveillance data in 14 countries and NRLs in 3 countries. The laboratories were involved in notifications in 16 countries and only in Luxembourg, the physicians were the only notifying partners. At the European level, the main

information flow is thus from the laboratories and/or physicians to the national public health institutes.

#### 2.1.2 Frequency of notification and forwarded data

In most of the countries, the data is forwarded to the national level continuously or weekly (table 4). The information about the notification frequency was not available from Greece. In 14 (88%) countries, the data is forwarded as single cases, when the current surveillance system in Germany is taken into account. This offers a good basis for collecting demographic data on the European level. Only in the Netherlands and Denmark, the data is forwarded in an aggregated form. In the Netherlands, *Campylobacter* isolates from faecal samples are reported weekly by fax from laboratories within the sentinel system. Austria receives the data both in an aggregated form (statutory notification system) and as single cases (sentinel surveillance system).

Demographic information (age, sex) is most frequently transmitted in both surveillance systems (table 5). Travel history is routinely forwarded in 9 countries, but information about outcome and symptoms is transmitted in 4 and 6 countries respectively. Laboratory confirmation (verified diagnosis) is known in 15 countries and differentiation on species level is transmitted routinely in 8 countries.

#### 2.1.3 Case definitions for surveillance

Ten out of 17 countries with surveillance systems have given case definitions for *Campylobacter* infection (table 6). In Denmark, Finland, Norway, Spain, Sweden, Switzerland and the United Kingdom the case definition is based on culture confirmed laboratory diagnoses regardless of clinical signs and the type of sample. Austria and Italy include the word "patient" to the case definition. Luxembourg includes symptoms in the case definition. In addition to a laboratory diagnosis, Denmark defines a 6 month and Finland 12 month time-period for a case.

Many countries defined certain criteria for reporting data on *Campylobacter* infections to the national level, rather than a formal case definition. In Germany, only the number of *Campylobacter* infections was reported from some Federal States until the end of 2000. Since 2001, a formal case definition based on laboratory confirmation was introduced. In France, the sentinel data is reported on the basis of laboratory confirmation. In Iceland, the reporting of *Campylobacter* infections to the central register is based on findings of both clinical symptoms and positive laboratory diagnostics.

#### 2.1.4 Reported Campylobacter infections in humans

Fifteen out of 18 countries reported 134 971 *Campylobacter* infections in 1999 (table 7). The data from Germany covered only 11 Federal States out of 16. No data were available from France, Italy and Portugal. Based on reported numbers, the notification rate per 100 000 inhabitants ranged from 2.9 in Greece to 166.8 in Iceland in 1999 (table 7). In 1999, the mean notification rate /100 000 inhabitants of European countries (n=15) was 70.7 (95% CI 55.5 – 89.6). In 1998, the mean notification rate was 61.3 (95% CI 46.7 – 78.4). The increase in mean notification rate was 16% between 1998 and 1999. The figures from 1998 and 1999 are comparable as for both years the data were available from the same countries and regions.

#### 2.1.5 Trends of Campylobacter infection in European countries

All data discussed here are presented in table 7.

In Austria, a steady increase in *Campylobacter* cases is seen between 1996 to 1999, ranging from 1131 to 3188 respectively. Both the statutory and the sentinel surveillance system were introduced in 1996, so an assessment for trend is limited.

Since the introduction of the sentinel surveillance system in Belgium in 1991, there was an increase of campylobacteriosis cases between 1995-1998 (4879 to 6610). The number plateaued in 1999.

In Denmark, there was an overall rise from 2601 to 4164 cases throughout the period 1995-1999. Human *Campylobacter* infections were made statutory notifiable in 1993.

England and Wales has seen a rise in cases from 43876 - 54994 in the four years from 1995-1998. The number decreased slightly in 1999. A sentinel surveillance system has been introduced in 2000.

The statutory surveillance was introduced in Finland in 1994. The figures have been stable at around 2500 infections between 1995 to 1997. The number of reported cases increased further in 1999.

In Germany, infections of *Campylobacter* have been statutory notifiable since 1979 as "other forms of infectious enteritis". The reports of *Camyplobacter* came only from 6-11 Federal States between 1995 and 1999. Therefore, the trend cannot be interpreted. *Campylobacter* has become statutory notifiable as a separate disease for the whole of Germany since 2001.

In Greece, the only official data source about human campylobacteriosis is based on notifications of isolates reported to the Ministry of Health from hospital laboratories. Data on isolates were only available for 1998-1999, thus trend assessment is limited.

In Iceland, statutory surveillance was introduced in 1999, so again an assessment for trend is limited. Incidences for the period 1995-1998 are based on reports from regional laboratories to a central register for infectious diseases.

Ireland has a sentinel surveillance system, which was introduced in the 1990s. There was a steady increase in *Campylobacter* cases reported to the co-ordinating institute from 1995-1999 (644-2085).

The listed data from Luxembourg is based on isolated strains reported to the Laboratoire National de Sante for the period 1995-1999. The number of reported isolates shows more or less stability for the 5-year period (range: 136-176). *Campylobacter* infections were made statutory notifiable in 2000.

In Northern Ireland, 301 additional cases were reported in 1999 compared to the figure for 1995 (858 versus 557).

In Norway, the figures were stable from 1995 through 1997 at approximately 1100 infections a year. A steep increase was reported from 1178 in 1997 to 1700 in 1998, with a further rise to 2027 infections in 1999. No larger outbreak has been reported since 1997, when a total of around 367 outbreak-related infections occurred.

Scotland reported an increase from 4377 to 6375 cases from 1995 through 1998. A drop to 5861 cases was seen in 1999.

In Spain, the sentinel surveillance system was introduced in 1989 and has collected data showing a steady increase from 3237 cases to 5191 cases of campylobacteriosis over the 5-year period. Spain states that the degree of coverage of their sentinel system is unknown. The total Spanish population was used for calculation of the incidence rates reported.

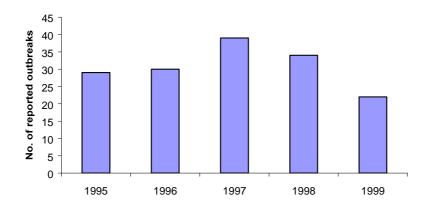
Sweden reported a small drop in the number of cases reported from 1995-1997 (5580 vs. 5306). An annual increase was seen in both 1998 and 1999 (6544 and 7137) respectively. The statutory notification system was introduced in 1989.

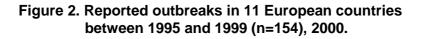
Switzerland had a minor decrease in the number of *Campylobacter* cases from 1997 to 1998. Apart from this small decrease in 1997, figures have been stable ranging from 5044 - 5455 infections from 1995 to 1998. An increase in reported cases was noted in 1999, with 6709 infections. Human campylobacteriosis was made statutorily notifiable in 1987.

The Dutch sentinel laboratory-based system which was established in 1995 has an estimated coverage degree of approximately 62%. The reported figures show minor fluctuations over the 5-year period; 2871 cases in 1995 with a maximum of 3741 cases reached in 1996. From 1997 through 1999, cases dropped from 3646 to 3135.

#### 2.1.6 Reported outbreaks

From 1995 to 1999, 11 countries reported 154 outbreaks. England and Wales reported 51, Sweden 36, Germany 20, Spain 16 and the rest of Europe reported 31 outbreaks. The highest number of reported outbreaks was in 1997 (figure 2). The reporting of outbreaks varied a lot by countries and the numbers presented here may not entirely reflect the real situation.





In 48% (74/154) of the reported outbreaks, food was the likely vehicle of transmission (including nine outbreaks where the source of infection was raw milk and food). For 15% (23/154) of the reported outbreaks, consumption of unpasteurised milk was reported as the source of infection, another 15% (23/154) were waterborne. In 21% (33/154) of the reported outbreaks, the cause remained unknown or was not reported.

An analytical epidemiological study (case-control or cohort study) was carried out in 22 of the 154 reported outbreaks (14%), in 7 (5%), a descriptive study was performed, but for the majority

of the outbreaks (124 or 81%), it was unknown or not reported whether an epidemiological study was done.

#### 2.2 National Reference Laboratories (NRLs) for Campylobacter

Laboratories that perform reference tasks (referred to in the following as NRL) for *Campylobacter* infections exist in 13 European countries. Eleven of these were national reference laboratories and two were other laboratories that perform reference tasks for *Campylobacter* (table 8). PHLS-CDSC provided the full data for England and Wales but it also collaborates with Scotland and Northern Ireland. In Germany, there is a consulting laboratory for *Campylobacter* in addition to the NRL.

In 7 countries (Austria, Belgium, Denmark, France, Germany, Luxembourg and the United Kingdom), the NRL has been officially assigned (table 8). In three countries, Greece, Iceland and Norway, the NRLs for *Campylobacter* infections were recommended. Recommendations were given by the Medical School at the University of Athens (Greece), the Ministry of Health (Iceland) and as an informal agreement among microbiological laboratories and the National Food Control Authority (Norway).

Three countries, Germany, Sweden, and Switzerland, reported having other laboratories performing reference tasks for human *Campylobacter* infections (table 8). Five countries, Finland, Ireland, the Netherlands, Portugal, and Spain, reported having no laboratory performing reference tasks. The Netherlands pointed out that RIVM (NPHI) and one other institute are involved in research projects on an ad hoc basis, but no institute is directly responsible for reference tasks.

#### 2.2.1 Tasks of National Reference Laboratories

In 13 countries, the NRLs perform one or more of the following tasks: confirm results (12), develop new typing methods (10), develop proposals for standardisation of methods (9) and new analytical methods (9), conduct training courses (7), and carry out routine primary diagnosis of specimen (7) (table 9). Less frequently, they examine official specimens from monitoring programs (5), co-ordinate methods for antibiotic resistance testing (4), provide reference material for diagnostic research and training (3), co-ordinate application of typing methods (3), and finally provide reference material for diagnostic laboratories (1) (table 9).

Only in Iceland and Luxembourg, the NRLs receive all the isolates from the country (table 10). The isolates are sent there within fixed time intervals. In 12 countries, the NRLs receive isolates of *Campylobacter* when there is a diagnostic problem at the sending laboratory. Austria, France, Iceland, Luxembourg and the UK receive all isolates from sentinel laboratories within a fixed time interval. When there is a suspicion of an outbreak situation, isolates are sent to the NRL in Austria, Belgium, France, Germany, Switzerland and the United Kingdom. Greece, Italy and Norway reported to receive isolates on an ad hoc basis.

#### 2.2.2 Campylobacter isolation methods

The principle of *Campylobacter* isolation was the same in all NRLs. The sample was cultured to selective agar plates and incubated at 37°C or 42°C in microaerobic atmosphere. In Sweden and Norway, the NRLs did not culture primary samples. The NRLs in Austria, Denmark, Germany, Iceland, Luxembourg and United Kingdom reported using mCCDA as a selective medium (table 11). Skirrow's medium was used in NRLs in Germany, Greece and Iceland. Belgium and Switzerland reported using Butzler's medium in NRLs. Furthermore, Germany reported using two other media resulting in a total of four different media. In France and Italy, Karmali's medium is used. The incubation temperature varied from only 37°C (8 NRLs) to 42°C (4 NRLs). Luxembourg and Belgium reported the use of both temperatures. Incubation time was mostly 2 days. All NRLs incubate the plates in microaerobic atmosphere but the methods to achieve it varied by NRLs (table 12). Commercial gas pack was used in six NRLs. Evacuation and replacement system was also used by six NRLs.

The NRLs in four countries, Belgium, France, Greece, and Italy, reported always using filtration for stool specimens (table 13). In four countries, the NRLs reported using enrichment procedures sometimes in specific situations like in outbreak investigations (United Kingdom), in case of Guillain-Barré syndrome (France) and for food samples (Germany, Luxembourg).

Ten NRLs out of 13 reported using various laboratory kept *Campylobacter* species for positive control growth on selective media to control for media specificity (positive growth on one type of media and negative on other) and sensitivity of investigated clinical samples or isolates. Most commonly used strains were *C. jejuni* (10 countries) and *C. coli* (8 countries) (table 14).

The NRLs used different conditions to store the isolates. The temperatures for storage of isolates varied mainly between  $-70^{\circ}$ C to  $-80^{\circ}$ C. The media and storage temperatures are listed in table 15.

Nine countries have given recommendations for primary *Campylobacter* isolation. These are presented in detail in part 4.2.

#### 2.2.3 Phenotyping of Campylobacter strains

Several methodologies have been applied to identify *Campylobacter* strains to the species level. Phenotyping refers to various metabolic activities expressed by an isolate and may include specific biochemical reactions, colonial morphology, and environmental tolerances, e.g. the ability to grow at extreme temperatures. The NRLs in 11 countries out of 13 used oxidase and catalase tests for phenotypic identification (table 16). Other commonly used tests for phenotypic identification (table 16). Other commonly used tests for phenotypic identification (table 16), indoxyl acetate hydrolysis (8 countries), direct microscopy (7 countries), and urease production (6 countries). Api Campy (a commercial test kit) is used in 6 NRLs. A range of two to five NRLs apply one or more of the following methods: H<sub>2</sub>S / Cysteine (Pb-acetate), nitrate reduction, Müller-Hinton broth plus 1,5% NaCl, Müller-Hinton broth plus 1% glycine, Mac Conkey agar or H<sub>2</sub>S/ PBP (Peptone broth phosphate). Also utilised are the media listed in parentheses: France (TSI, Triple sugar iron, separate or with DNAse), Iceland (Müller-Hinton Broth), Luxembourg (Nitrite reduction), Norway (TSB, Trypticase soy broth). The atmospheric compositions varied for the different tests used, as well as the temperature and length of incubation. The NRLs in Belgium, France, Germany, Italy and Norway used the phenotyping scheme of Lior.

Bacteriophages are viruses capable of infecting and lysing bacterial cells. Among species for which numerous lytic bacteriophages have been identified, strains can be characterised by their susceptibility to a standard set of phages. Phage-typing scheme according to Preston was used only in UK.

Serotyping is based on antigenic determinants expressed on the cell surface. The NRLs in Germany and United Kingdom used the modified serotyping method of Penner and they produced their own antisera. In Greece, the Penner serotyping scheme with commercial antisera was used. In Denmark, a sub-sample of strains is serotyped at the NRL on the veterinarian side annually.

Antimicrobial resistance patterns were used in 8 NRLs for phenotyping (table 17). Enzyme profile auxotyping was used in France only and total fatty acid gas chromatography in United Kingdom only.

#### 2.2.4 Molecular sub-typing of Campylobacter strains

Since phenotyping techniques have not enough discriminatory power and the reagents are of limited availability, DNA-based approaches have emerged as the preferred methods for the strain subtyping. Two main categories of genotyping techniques were used for *Campylobacter* in the 13 NRLs in European countries surveyed in the first study. Direct DNA-based analyses of chromosomal or extrachromosomal genetic elements were performed in 9 countries and PCR-based profiling was performed in 7 countries (table 18). The most common genotyping method was PFGE, which was used in 8 countries.

#### 2.2.5 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was used for two different purposes: either to choose an appropriate antimicrobial agent for patient treatment or to differentiate the species. Usually, the susceptibility for nalidixic acid and cephalotin were used for species differentiation while the susceptibility for ciprofloxacin and erythromycin were clinically relevant. The NRLs reported three different methods that were used for susceptibility testing; agar diffusion, E-test, and agar dilution.

The NRLs in 12 countries reported performing antimicrobial susceptibility testing for *Campylobacter*. Several NRLs used more than one method for susceptibility testing. In France and Germany, the NRLs only used agar diffusion method routinely (table 19). In Luxembourg, E-test was the only method used at random intervals and Norway used only E-test routinely. Both E-test and agar diffusion were used in Austria, Belgium, Denmark, Iceland, Italy and Switzerland. All of them except Iceland used agar diffusion routinely. In Iceland, agar diffusion method was only used for species differentiation and E-test was used routinely for ciprofloxacin and erythromycin. In Belgium, E-test was used routinely in Greece. Agar dilution was performed routinely in the United Kingdom and occasionally in Greece. In five countries (Austria, Denmark, Italy, Switzerland, and United Kingdom), E-test was performed especially for ciprofloxacin and erythromycin to confirm unclear results of agar diffusion.

In the NRLs, most frequently tested antimicrobial agents were nalidix acid (12) and erythromycin (11). Other frequently tested agents were ciprofloxacin (9) and cephalotin (8) (table 20).

#### 2.2.6 Quality assurance and accreditation

Internal quality assurance means procedures that are decided by the laboratories themselves to control different steps during the analyses. The NRLs in six countries, Denmark, Germany, Iceland, Luxembourg, Switzerland, and the United Kingdom have internal quality assurance procedures (table 21). The reported procedures were media control (Denmark, Iceland) and weekly resistance of reference strains and monitoring the growth conditions (Luxembourg).

External quality assurance (EQA) means quality testing procedures that are organised by an external agent, which may be a company, university, institute, or any other institution that provides controlled material for quality control testing. External quality assurance can also be organised between laboratories if suitable commercial material is not available. Four countries, Denmark, Germany, Iceland, and Norway, use external quality assurance for their diagnostic performances where one or more of the following methods are included in the system: detection of agents in a sample (all four countries); detection of antibodies in a sample (Iceland); typing of investigated isolates (Denmark, Iceland, Norway); antibiotic resistance testing (Denmark, Iceland). NRLs in six countries plan to adopt an external quality system (table 21).

Accreditation, based on internationally agreed criteria, is a procedure by which an authoritative body gives formal recognition that a body or person is competent to carry out specific tasks. At the European level within EA (European Co-operation for Accreditation) an active cooperation between national accreditation bodies has successfully led to European multilateral agreements (MLA) almost in all fields of accreditation. In testing laboratories, accreditation is test specific. Certification is a commercial action by a third party, demonstrating that adequate confidence is provided that a duly identified product, process or service is in conformity with a specific standard or other normative document.

The NRLs in Germany and Switzerland had an accreditation for *Campylobacter* cultivation according to standards EN 45001/ ISO Guide 25 or ISO 17025, a recently approved standard where the standards EN 45001 and ISO Guide 25 are combined. In Denmark, an accreditation was in process, and the United Kingdom reported having a Clinical Pathology Accreditation, which was not test specific (table 21).

# 3. Results of the second Survey (Survey II)

A total of 1014 primary laboratories from 10 EU countries responded to the second survey. Information about non-responders was available from all countries except Germany (table 22). In England & Wales, none of the laboratories that belonged to the National Health Service (41%) responded to the survey. The sampling methods in Austria, France, Germany, Ireland, Italy, Portugal and Scotland reached a larger group of laboratories not all of which performed *Campylobacter* diagnostics. In Denmark and Finland, the questionnaires were targeted directly to *Campylobacter* laboratories and the responding laboratories (64% and 84% respectively) represented well the primary *Campylobacter* laboratories. A total of 622 (61%) laboratories reported performing *Campylobacter* diagnostics. Most of them were either hospital-based (53%) or private (41%) (table 23). In the group "other", almost all *Campylobacter* laboratories were in universities, only one laboratory was in the army (Germany). In Germany, one laboratory reported performing antigen-test (Virotech) for stool samples.

The following results concern only the 622 laboratories that perform primary microbiological *Campylobacter* diagnostics and the words "primary laboratory" or "*Campylobacter* laboratory" refer always to these laboratories.

#### 3.1 Patient characteristics

Most of the laboratories (61%) report serving outpatients (table 24). About 40% of laboratories serve patients from major hospitals and primary care hospitals each. One laboratory may serve more than one group of patients.

From demographic data, sex and age were most frequently transmitted to laboratories with sample submission (table 25). In Denmark, Finland and Scotland, the data about age and sex was always transmitted to laboratories. Two thirds of laboratories (68%) received the information about residence (table 25). About half of the laboratories got the information about history of diarrhoea and one third received information about hospital admission (table 26). Only 11 % of laboratories received the date of symptom onset, but 83% received the date of specimen collection (table 26). Twenty percent of laboratories (range 5% to 50%) got the information of connection to an outbreak and 25% (range 13% to 90%) received travel information (table 27). Of all surveyed countries, travel data was most frequently transmitted in Finland and England & Wales where 90% and 83% of laboratories received history of travel , although this information may not be complete.

#### 3.2 Campylobacter investigations

The size of laboratories was assessed on the basis of the total number of stool samples. Of 622 laboratories, 578 (93%) provided information about stool samples in 1999. Small laboratories (< 1000 stool samples in 1999) covered 42% of all surveyed laboratories but there were no small laboratories in Denmark and England & Wales (table 28). In Denmark, the investigations were centralised in big laboratories. In France, most replying laboratories (89%) were small. Among all countries, total of 74 (13%) laboratories reported to have had > 10 000 stool samples in 1999. Only three laboratories reported to have had > 100 000 stool samples in 1999. One laboratory was in Austria and two laboratories in Germany.

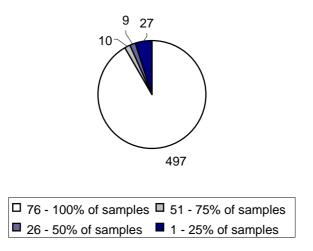
Overall, the total number of *Campylobacter* investigations was about half the number of all stool samples (table 29) but the ratio varied by countries. In Austria, Finland, France, Germany, Ireland, Italy, and Portugal, the ratio between incoming stool samples and performed *Campylobacter* investigations was about 2:1 meaning that about every second stool sample was cultured for *Campylobacter*. In Denmark, England & Wales, Greece, and Scotland the ratio was about 1:1.

A total of 484 (78%) laboratories reported positive *Campylobacter* results (table 29). The mean of positive isolates / 100 investigations (isolation rate) ranged from 2.3 to 6.2 between countries (table 29). This indicates that the routine in taking samples and/or the methods to investigate *Campylobacter* differed between countries and laboratories. In countries where almost all stool samples were investigated for *Campylobacter*, the laboratories were more likely to have an isolation rate > 4.00 (OR 4.4, 95% Cl 1.6 – 12.1). This analysis was performed only with the laboratories that had reported exact numbers for stool samples and *Campylobacter* investigations (n=216). Two thirds of laboratories (67%) reported to have had 1 - 99 *Campylobacter* findings in 1999 (table 30). Eight laboratories reported to have had > 1000 positive samples in 1999, one laboratory was in Denmark, one in England & Wales, and six were in Germany.

The proportion of laboratories that cultured the samples routinely for *Campylobacter* ranged from 14% in Finland to 100% in Denmark (table 31). These proportions were in concordance with the noted ratio between total number of stool samples and performed *Campylobacter* investigations in all countries except in Ireland. This is explained by the low number of laboratories that had reported the number of *Campylobacter* investigations compared to the laboratories that had reported the number of stool samples. Every fifth laboratory (20%) reported performing the culturing based on the information that samples are related to

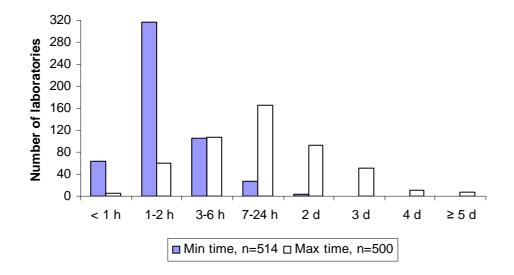
outbreaks, which is well in concordance with the fact that only 21% of laboratories receive the information of an outbreak connection (table 27). Almost half of the laboratories (45%) reported investigating *Campylobacter* based on a request on the submission form. In Finland, 86% of laboratories reported to culture for *Campylobacter* if it is requested on submission. This is due to the standardisation of testing algorithms. In France, 61% of laboratories reported culturing *Campylobacter* when blood or mucus was present in the stool sample. About half of the laboratories in France (48%) and Germany (45%) reported having other instructions for culturing. In France, the most frequent specifications were liquid stools, diarrhoea and/or infants' stools. In Germany, the specification was mostly a general request of pathogenic microbes.

The laboratories received stool samples in different forms. Four sample types were specified in the questionnaire, stool samples with and without transport medium, and rectal swabs with and without transport medium. Most laboratories (543/622) reported receiving all or part of the stool samples without transport medium. Of these laboratories, 497 (91%) reported receiving 76-100% of all stool samples without transport medium (figure 3). This means that the time interval between sample taking and the beginning of the investigation becomes very important. Stool samples or rectal swabs in transport medium were submitted to 24% (148/622) of laboratories but the annual proportions of these sample types varied by laboratories.



# Figure 3. Primary laboratories receiving stool samples without transport medium in 10 European countries (n = 543), Survey II, 2000.

Most laboratories (87%) reported culturing the samples for *Campylobacter* within 24 hours. However, seven laboratories reported a maximum time interval  $\geq$  5 days and one of them even 20 days between sample collection and culturing (figure 4). Taking into account that most laboratories received the samples without transport medium, such time intervals between sample collection and investigation are very long, even if the transport medium would be used. Many laboratories reported the minimum time interval of < 1 hour which does not sound reasonable. It is probable that some laboratories thought of the time interval between the sample arrival and culturing.



# Figure 4. Minimum and maximum time intervals between the sample collection and culturing for *Campylobacter* in 10 European countries, Survey II, 2000.

#### 3.3 Method description

Over half of the laboratories (330/583) reported using a nationally or internationally published method (table 32). Some of these laboratories (28/330) did not specify the method further. The proportion of laboratories using a published method ranged from 23% in Portugal to 100% in Greece and England & Wales. One quarter of laboratories (24%) didn't know whether their method was based on published documents or not.

#### 3.4 Pre-culturing procedures

For *Campylobacter* investigations, direct microscopy for stool samples was not widely used in European laboratories. In France, most laboratories (88%) used direct microscopy, and 74% (160) used it always (table 33). In Italy, one in three laboratories and in Greece four of the six laboratories reported using direct microscopy always or sometimes. In Denmark and Ireland, this method was not used at all.

Filtration was even less frequently used. Only 5% of all laboratories reported using filtration (table 33). Sedimentation was in use in 16 laboratories, of which one was in Denmark, 2 both in France and Germany, 10 in Italy and one in Scotland (table 34). Commonly, the laboratories suspended the sample in saline or peptone water and allowed it to sediment few minutes before plating it on agar plates. One laboratory reported suspending the sample in selenite broth which is generally used for salmonella enrichment. A total of 70 laboratories (11%) reported always using enrichment procedure (table 34). Of those, 51 (73%) were from France and 10 (14%) from Italy.

#### 3.5 Culturing methods

Laboratories used many different media for culturing *Campylobacter* (table 35). In Germany and Italy, the variation of media used was the largest. In some countries, a major media type could be clearly pin-pointed. In France, Campylosel-agar was widely used. In Denmark and Finland, mCCDA was most frequently used. In most laboratories, the sample was cultured on a selective medium/media and incubated at 37°C or 42°C for 48-72 hours (table 36). Only one laboratory reported the incubation temperature of 39°C. The higher incubation temperature allows only thermophilic or thermotolerant species to grow (*C. jejuni, C. coli, C. upsaliensis, C. lari*) being at the same time a selective factor. If the lower temperature is used, more species are found (e.g. *C. fetus*), but at the same time other bacteria, e.g. *Arcobacter spp.* may grow on the plate. A commercial gas pack were mainly used (86% of laboratories) to create the microaerobic atmosphere (table 37).

Most laboratories (82%) cultured only one sample per agar plate (table 38). However, as many as 108 laboratories reported culturing more than one sample on one agar plate (usually two samples). This practice was reported in Germany by 41 laboratories and in Austria by 30 laboratories. In Germany, 32 (78%), but in Austria only 7 (23%) of these were private laboratories.

Almost all (94%) laboratories reported confirming the suspected colonies. The two most frequently used methods for confirmation were microscopy (98%) and oxidase test (90%) (table 39). Two third of laboratories (64%) reported using catalase test. In England & Wales, this method was not in use in any of the laboratories which replied (n=6). Overall, latex agglutination tests were not widely used, only 11% of laboratories reported such a test for confirmation. In Italy, latex agglutination was in use in 31% of laboratories. In Austria and Ireland, about every fourth laboratory used latex agglutination test for confirmation. Of other confirmatory tests, the most frequently reported t was the commercial biochemical test kit API Campy. Other reported

tests for confirmation were testing the susceptibility for cephalothin, growth at 25°C and 37°C and aerobic control for growth.

Over half of the laboratories (59%) identify the isolates further to the species level (table 40). However, laboratories from England & Wales reported that they never determine the species of the *Campylobacter* isolates. Species determination is not a common procedure in Denmark either; only one laboratory reported using a PCR-based method sometimes. Most frequently used methods were hippurate hydrolysis (59%) and susceptibility for nalidixic acid (60%). Nitrate reduction, indoxyl acetate and H<sub>2</sub>S production were in use in 10% - 18% of laboratories. Other methods used for species determination were cephalothin susceptibility, API Campy and growth at 25°C and 37°C. In Finland and Scotland, all laboratories tested at least for hippurate hydrolysis. In Greece, all laboratories tested at least for susceptibility to nalidixic acid.

Very few laboratories (15%) stored the *Campylobacter* isolates routinely (table 41). The isolates from outbreaks were stored even less frequently (5%). About half of the laboratories (47%) did not store the isolates at all. In Greece, 5/6 (83%) laboratories reported storing isolates routinely. In France, 73% of laboratories reported storing isolates sometimes. In Scotland 83% and in Denmark 86% of laboratories reported not storing the isolates. Among those laboratories (n=155) who reported the storage time, 58% reported storing the isolates for years (table 42).

#### 3.6 Antimicrobial susceptibility testing

About half of all *Campylobacter* laboratories (46%) reported always performing antimicrobial susceptibility testing (table 43). Almost as many (44%) reported not testing for antimicrobial susceptibility. In Greece, all laboratories always tested for susceptibility whereas in Portugal, 92% of laboratories did not test the susceptibility of *Campylobacter* for antimicrobial agents.

Agar diffusion method was used for susceptibility testing by 85% of laboratories (table 44). Agar dilution and E-test method were used only by 6% and 1% of laboratories respectively. Three laboratories reported using one of the following methods; Vitek Card, Sceptor and Stokes Disc. One laboratory had an automatic system. Müller-Hinton agar with or without blood was used in 77 laboratories (n = 206, 37%) for the agar diffusion method and 32 laboratories (16%) used blood agar. The incubation temperature for agar diffusion method was either  $37^{\circ}C$ (146/280 laboratories, 52%) or 42°C (122/280, 44%).

The antimicrobial agents tested varied a lot between countries and laboratories. Laboratories most frequently tested the susceptibility of *Campylobacter* for erythromycin (92%) and

ciprofloxacin (83%) (table 45). Other frequently tested agents were tetracyclin (64%), ampicillin (59%), cephalotin and gentamycin (both 56%). Many laboratories also reported the susceptibility testing for amoxycillin + clavulanate, tobramycin, trimethoprim + sulfa and clindamycin to mention a few.

#### 3.7 Information flow

#### 3.7.1 Sending the isolates

Very few laboratories sent their *Campylobacter* isolates routinely for further characterisation or confirmation (table 46). National Reference Laboratories (NRLs) received relatively few samples from local laboratories. Of all laboratories which replied (615/622), 31 (5%) reported sending the *Campylobacter* isolates always and 51 (8%) occasionally to the NRL. About as many laboratories reported sending the isolates to laboratories other than the NRL. In England & Wales, 5/6 laboratories (83%) reported sending the isolates always to the NRL. About one-third of laboratories in Denmark (29%), Finland (33%) and Ireland (29%) occasionally sent the isolates.

#### 3.7.2 Reporting the findings

Few laboratories reported positive findings directly further to the National Surveillance Centre (10%) or the NRL (4%) (table 47). In Denmark, England & Wales, and Finland, over 80% of laboratories reported directly to the NSC. The reporting proportion to the NSC is also relatively high in Scotland (74%) and in Greece (67%). In other countries, only 0-7% of laboratories reported directly to the NSC. In Germany, 91% of laboratories reported to local health authorities as required by law. In France, most laboratories (93%) replied that *Campylobacter* is not a reportable infection.

#### 3.8 Internal quality assurance

Internal quality assurance was differently formulated in the survey on primary laboratories. As it includes various procedures and practices, only the most important quality assurance factor relevant to the sensitivity of method was asked for in detail, i.e. quality control of agar plates. The routine for controlling the quality of agar plates varied from 14% (30) in French laboratories to 100% (21) in Finnish laboratories (table 48). The large range in performing quality control for agar plates was mainly explained by the fact that many laboratories bought the agar plates and thus relied on the quality control that had been performed by the manufacturer. In France, only 5 % of laboratories reported preparing the agar plates themselves whereas the respective

percentage was 95% in Finland. Laboratories that prepared the agar plates themselves also controlled the quality of plates. The correlation between always preparing the agar plates and always performing quality control was  $r_s = 0.88$  (Spearman's correlation coefficient, p<0.01).

The storage time for self prepared plates varied from 1 day to 90 days (figure 5) and the median was 14 days. Another frequently reported storage time was 7 days. *Campylobacter* selective plates include usually two-three different antimicrobial substances and the plates can not be stored for a long time without an effect on sensitivity and selectivity whether they are self-prepared or bought. For example, one manufacturer of mCCDA agar recommends the storage time for prepared agar plates up to 7 days at 2-8°C in dark (Lab m).

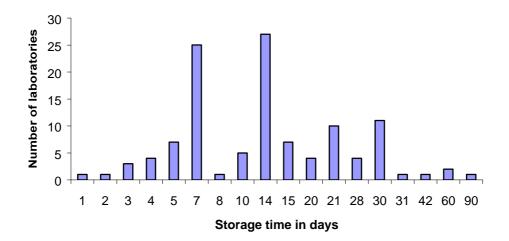


Figure 5. The storage time of self prepared *Campylobacter* agar plates in primary laboratories in 10 European countries (n = 115), Survey II, 2000.

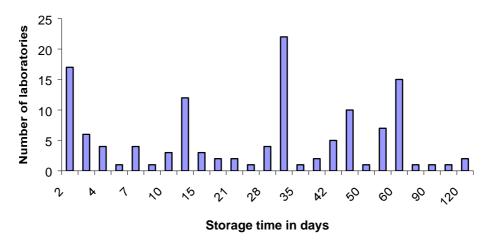


Figure 6. The storage time of bought *Campylobacter* agar plates in primary laboratories in 10 European countries (n = 128), Survey II, 2000.

Laboratories that bought the agar plates reported the storage times from 2 to 120 days and the median was 30 days (figure 6).

#### 3.9 External quality assurance (EQA)

External quality assurance (EQA) means a service whereby participating laboratories are sent samples on a regular basis which they test as if they had come from patients. Results are returned to EQA centres which provide a report that compares the participant's performance with that of all laboratories and/or groups of laboratories using the same test method(s). At the European level, almost half of the primary laboratories (44%) reported participating in the EQA schemes for *Campylobacter* culturing in their country (table 49). In Denmark, Finland, Scotland, and England & Wales, all laboratories that replied in the survey reported the participation in external quality assurance testing for *Campylobacter* culturing. However, many laboratories in France (58%), Germany (50%) and Italy (42%) were not sure if there were EQA schemes available in their country (table 50) which clearly indicates that local laboratories need more information about the EQA schemes available in their country and Europe.

Only a few laboratories (6%) reported having accreditation for *Campylobacter* culturing in Europe (table 51). In Scotland 78 %, and in England & Wales 50 % of laboratories had the Clinical Pathology Accreditation (CPA), which is a general accreditation for the laboratory and not method-specific. In Finland, every fourth laboratory (24 %) reported having an accreditation for *Campylobacter* culturing given by the national Finnish accrediting body FINAS (The Finnish Accreditation Service). Eleven laboratories (8 %) in Germany reported having an accreditation for *Campylobacter* culturing. Two of these were given by the College of American Pathologists

(CAP), 7 by Deutsche Akkreditierungsstelle Chemie GmbH (DACH), and 2 by Zentralstelle der Länder für Gesundheitsschutz bei Medizinprodukten (ZLG). DACH and ZLG are national German accrediting bodies. One laboratory in Austria and 7 laboratories in Italy reported having certification according to ISO 9000.

## 4. Combined results of both surveys

#### 4.1 Reported cases and primary laboratory findings

Of ten countries participating in the second survey on primary *Campylobacter* laboratories, 5 had statutory and 4 sentinel surveillance systems. One country (Portugal) had no surveillance system at the time the survey was conducted. Austria had actually both systems but in this section it has been grouped together with the countries that have statutory surveillance systems.

To roughly assess the information flow from primary laboratories, a comparison of notified cases with annual positive laboratory results by countries was made. It should be kept in mind that not all laboratories had reported their positive findings and not all laboratories in countries had replied to the survey and therefore the total number of *Campylobacter* laboratory findings / countries is more or less an underestimation of all *Campylobacter* findings in a country. Among countries with statutory surveillance system, primary laboratories in Austria, Denmark, Finland and Greece reported more *Campylobacter* findings than there were notified cases (table 52). This may be due to the fact that laboratories investigate follow-up samples from the same patients and these findings were included in the total number of primary laboratory findings and/or all cases were not reported. In Austria, only physicians were involved in the statutory notification system and the large difference between the primary laboratory findings and the notified cases indicates that over half of laboratory confirmed cases did not enter the surveillance system. In Germany, physicians notified the cases until 2001 but the information for 1999 comes only from 11 Federal States out of 16 and thus does not represent the whole country.

Among countries with sentinel surveillance systems, primary laboratories in Ireland, Scotland and England & Wales reported less *Campylobacter* findings than there were notified cases (table 52). This is explained by the fewer number of laboratories giving the information about their positive results. The information about notified cases was not available from France and Italy. In France, the sentinel system concerns only some hospital laboratories with more severe cases and thus does not reflect the incidence of Campylobacter infection in the general population.

As sentinel systems did not catch as many cases as statutory systems, the notified cases from these two systems were not comparable. However, if the sentinel system takes into account the population under surveillance, the incidence could be estimated for the whole country thus making the incidences between countries more comparable.

# 4.2 Recommendations for *Campylobacter* isolation by NRLs and the practices in primary laboratories

Nine countries with a NRL had given microbiological recommendations for routine bacteriological diagnosis of *Campylobacter* infections (Austria, Denmark, France, Germany, Iceland, Italy, Norway, Sweden, United Kingdom) (table 53). Nine NRLs reported having developed proposals for standardisation of methods (see 2.2.1). There was no difference in the use of published methods or recommended guidelines in primary laboratories between the countries with or without NRL (Wilcoxon t-test, p>0.1) indicating that though an official NRL does not exist in a country, the laboratories used published methods or guidelines.

# 4.2.1 Pre-culturing and culturing procedures

The following comparisons have been made within the framework of information from both surveys and therefore not all countries are included. A total of six countries with nationally recommended methods had information from both the national and local levels, so the comparison of procedures could be made for these countries.

The recommendations to use diagnostic procedures *before* specimen cultivation (pre-culturing procedure) varied by NRL. In France and Germany, enrichment was recommended when the expected amount of bacteria in a sample was low such as healthy carriers or prolonged time between sample collection and the start of investigation (table 54). Among primary laboratories, an enrichment procedure was routinely used in 51 (24%) of French and in 5 (4%) of German laboratories. In France, 160 (74%) laboratories reported performing direct microscopy for stool samples routinely. Austria and England & Wales did not have recommendations for procedures before cultivation, and none of the primary laboratories reported using any procedure before cultivation either. In Italy, pre-culturing procedures were not commonly used. Fifteen (13%) laboratories used direct microscopy and 10 (9%) laboratories used enrichment.

In the NRLs and primary laboratories, the general procedure for isolation was direct plating on a selective medium and incubation at 37°C or 42°C in a microaerobic atmosphere for 48 hours (table 55). The primary laboratories used many different selective media. Most diversity in selective media was found in Austria, Germany, and Italy, where primary laboratories reported using at least a total of 8-9 different media. Usually laboratories used one type of selective medium but some laboratories reported using two-three different media for one sample, and some reported incubating the plates both at 37°C and 42°C.

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Austria had given a choice of a selective medium and primary laboratories reported using at least eight different media. Campylosel-agar was the mostly used medium by primary laboratories in Austria and France (table 55). In Denmark, all primary laboratories reported using the recommended procedure. In France, Karmali's medium was recommended but only 10% of primary laboratories used it. The incubation times also tended to be longer than recommended 2 days. In Germany, five different media were quite equally used, mCCDA (17%), Butzler's medium (17%), Karmali's medium (16%), Skirrow's medium (15%) and CampyBap (14%). In Germany too, the incubation times tended to be longer than recommended. In Italy, the choice of selective medium was left to primary laboratories. The two most frequently used media were Karmali's medium (23%) and CampyBap (17%) and incubation times tended to be longer than recommended. In England & Wales, two most frequently used media were mCCDA (33%) and Preston (33%) but the interpretation has to be cautious due to information only from few (6) laboratories.

Overall, the primary laboratories used methods which were well within the frame of recommendations. The principle of the isolation method was the same in all countries. The use of different incubation temperatures (37°C and/or 42°C) may result in different isolation rates (see 3.5). Some laboratories reported incubation times as long as 168 h (7 days) which is unnecessarily long.

# 4.2.2 Confirmation of Campylobacter strains

Most primary laboratories (94%, table 39) in 10 European countries reported performing confirmation for isolated strains. For the six countries with information both from the national and local levels, the practices for confirmation are presented in table 56. The most commonly used tests for confirmation among primary laboratories in these six countries were microscopy (95%-100%), oxidase test (67%-100%) and catalase test (0%-86%). All of these tests were recommended in Austria and primary laboratories used all these tests except the catalase test which was used by only 54% of laboratories. In France, Italy and England & Wales, most primary laboratories performed the confirmation tests within the frame of recommendations. Denmark and Germany had not specified the recommended tests for confirmation in their guidelines. Some laboratories in Austria, France, Germany and Italy reported using information about resistance for confirmation.

#### 4.2 3 Antimicrobial susceptibility testing

Agar diffusion method was routinely used for antimicrobial susceptibility testing in most NRLs and primary *Campylobacter* laboratories (table 57). In England & Wales, the NRL used the E-test and agar dilution but none of the primary laboratories reported using these methods.

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However, there were only few laboratories participating in the survey and the results must be interpreted cautiously. Agar dilution was rarely used by NRLs and primary laboratories.

# 5. Conclusions

Almost all countries (17/18) had either statutory or sentinel *Campylobacter* surveillance systems which provides a good basis for international surveillance. As primary laboratories didn't send isolates or report findings regularly to the NRLs, the European-wide surveillance would be best organised through national surveillance systems. There exists, however, considerable variation in case detection between countries.

Most countries received the notifications as single cases which makes the data management and analysis more efficient. Almost all countries also received the basic demographic information about age and sex. Travel history information was also received in many countries. National surveillance systems also received information about laboratory confirmation in almost all countries.

Although the reported number of outbreaks has declined since 1997, it may not reflect the actual situation as so few countries systematically collect data from outbreaks. However, the total number of reported cases is increasing year by year in many countries.

Many NRLs developed proposals for standardisation of methods. The culturing methods in primary laboratories and NRLs have the same principle but they varied in details between NRLs and primary laboratories within and between the countries. This almost certain leads to variation in the case detection between laboratories. Standardisation and harmonisation of microbiological procedures is, therefore, urgently needed. In many countries, the interaction between primary laboratories and NRLs could be improved. Many laboratories are not aware of recommended guidelines given by NRLs in their country. Very few laboratories also sent the isolates to NRLs for confirmation or further characterisation.

Antimicrobial susceptibility testing methods were basically the same in most countries both on national and local levels and this offers a good basis for comparative surveillance of antimicrobial patterns.

# 6. Recommendations

The participants at the final meeting concluded that there is an appropriate basic infrastructure for a *Campylobacter* surveillance network and the following points should be considered in the preparation of such a network:

# 6.1 Why is a surveillance network for Campylobacter needed?

The reported cases of human Campylobacter infections are increasing in many countries revealing that these infections are emerging. However, the epidemiology of these infections is still incompletely understood. There is not enough information about the risk factors and the burden of the disease in different countries. Furthermore, this information is not collected and analysed centrally at the EU level. The economic impact in relation to other enteric diseases has not yet been assessed. Surveillance contributes to develop hypotheses on risk factors and to implement targeted studies to test their relative importance in countries. As Campylobacter is mainly transmitted via food and water, it has a potential to cause international outbreaks. Furthermore, travelling has been shown to be one of the risk factors for contracting the infection. International co-operation offers better tools for prevention of infection. Campylobacteriosis is also included in the list of diseases of a European surveillance system, which emphasises the need for EU-wide surveillance of human Campylobacter infections. A European surveillance network for campylobacteriosis is needed because it is unknown why the reported numbers of cases are increasing in many European countries and the rapid international movement of people and food enables large outbreaks to take place potentially affecting a large number of people in many countries.

# 6.2 Microbiological issues to be considered

Some countries have standardised the methods for isolation and species determination, but even so methods vary between the countries. Standardisation of sample handling is a critical factor for the sensitivity and specificity. An appropriate way to achieve general consensus between countries, and to create European recommendations and standard operational procedures should be sought. This could be achieved through a comprehensive collaboration of microbiologists in research (CAMPYNET) and on national levels (reference/support laboratories). The information flow from national to local level and vice versa could be improved in many countries. This would also provide the opportunity for stronger support for the primary laboratories from the NRLs. It is important to have at least one laboratory (NRL or other) performing reference tasks in each country to support local laboratories and develop method standardisation, which in turn improves the case detection. NRLs could collect the strains in a

systematic manner. This would ensure a strain collection to be available for molecular or serologic sub-typing on the EU-level.

Since one common standardised molecular sub-typing method cannot currently be recommended for epidemiological use on a large scale, collaboration with the EU-funded working group, CAMPYNET is highly recommended. This would offer all NRLs and other central laboratories the possibility to assess and develop their molecular typing methods. This would enhance the ability for the surveillance network to detect international outbreaks. However, the final decision of the sub-typing method (serologic / molecular) should be left to the network group, appropriate expert group and the microbiologists at national levels. When a suitable molecular sub-typing method is available, a priority list should be established which isolates should undergo molecular subtyping (e. g. isolates from suspected outbreaks, or routinely a all cases from a certain area for detection of diffuse outbreaks within this area).

Antibiotic resistance testing is an important part of the surveillance. The survey for local laboratories showed that about half of the laboratories perform routinely antimicrobial susceptibility testing. It is reasonable to include the surveillance of antimicrobial resistance into the European network. The collaboration with EARSS and other EU-funded actions should be explored.

Quality assurance for culture and antibiotic resistance testing should be encouraged and the effective dissemination of the information about the available schemes to local levels should be encouraged. The supportive role of NRLs is not very clear for primary laboratories and the contacts between NRLs and primary laboratories should be strengthened.

# 6.3 Epidemiological issues to be considered

Surveillance systems should be implemented so that trends and effects of intervention measures can be monitored. If there is a change, Member States should be encouraged to record, evaluate and communicate consequences of an change to the network. For sentinel surveillance systems, countries should be encouraged to assess the level of coverage to achieve comparability in incidence calculations.

Population-based studies should be undertaken to assess the true burden of *Campylobacter* infections. If this were done in a Europe-wide study, comparisons between countries could be made.

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When European recommendations for sample handling, culture procedures and molecular subtyping are available, a EU-wide case-control study on risk factors for *Campylobacter* infections could be considered.

Travel is one of the most important factors to be included in the surveillance systems. Countries should be encouraged to include travel information in their surveillance systems.

# 6.4 Steps to be taken

### 6.4.1 Objectives

The surveillance network would have the main objectives to provide comparative data on trends within and between countries and to also recognise EU-wide outbreaks among apparently sporadic cases. Currently, the data are not comparable and conclusions from comparisons between countries can not be made. Other objectives would be to generate hypotheses about risk factors and to encourage the investigation of these hypotheses. Thus, the surveillance network would contribute to the scientific basis for the prevention and control of campylobacteriosis.

#### 6.4.2 Initial network

The European surveillance on human campylobacteriosis could be initiated with the information now available and could be developed step-wise over time. To achieve this aim, a EU-wide surveillance system should be developed using the common case definition and a minimum data set (age, sex, travel, laboratory information) that has already been developed by the Community Network under Decision N°2119/98/EC which would be improved over time. The network could start with a sentinel system of few laboratories in each country with a known population coverage. The Member States should be encouraged to establish a support/reference laboratory for Campylobacter. The network would aim at detecting international outbreaks when European recommendations about molecular subtyping of *Campylobacter* are available. Although the commonly used molecular typing method is yet to be determined, the preparatory work for the network should be started already.

# 6.4.3 Interaction with other EU-programs

In collaboration with the Community Reference Laboratory for the Epidemiology of Zoonoses, the ways to compare the data from humans, animals, feed and food stuff should be sought.

To include the surveillance for Campylobacter antimicrobial resistance in the network, the collaboration possibilities with EARSS should be sought.

To gain the maximum benefit of a surveillance network, the information about outbreaks e.g. the number of outbreaks and likely number of ill could also be collected centrally. As a continuous discussion between research and surveillance is necessary, this would offer an appropriate forum for interaction between these parts. Furthermore, the interaction and discussion between microbiologists, veterinary microbiologists and epidemiologists is needed. Although the CAMPYNET project is due to end this year a further proposal is in progress to maintain this highly successful network. Part of this proposal will be to develop recommendations for the use of molecular typing methodologies for epidemiological purposes. It is anticipated that this would require input from the Campylobacter Surveillance network once it is established. The procedures could be similar to Enter-net network.

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Annex 1: Tables

Survey I			Sur	vey II		
Participating countries	Total sent	Number of respon (response		Number of <i>Campylobacter</i> diagnostic laboratories among responded labs (% by country)		
Austria	58	44	(76)	41	(93)	
Belgium						
Denmark	11	7	(64)	7	(100)	
Finland	25	21	(84)	21	(100)	
France	500	245	(49)	217	(89)	
Germany	1430	450	(31)	138	(31)	
Greece	15	6	(40)	6	(100)	
Iceland						
Ireland	53	49	(93)	35	(71)	
Italy	192	132	(69)	115	(87)	
Luxembourg						
The Netherlands						
Norway						
Portugal	130	29	(22)	13	(45)	
Spain						
Sweden						
Switzerland						
UK, Scotland	38	25	(66)	23	(92)	
UK, England & Wales	35	6	(17)	6	(100)	
TOTAL	2487	1014	(41)	622	(61)	

 Table 1. Participation and responses in the two Campylobacter surveys, 2000.

Table 2. Sources for data (year of introduction) about <i>Campylobacter</i> infections in	
European countries (n =18), Survey I, 2000	

	Statutory notification	Sentinel system	Other sources of data
Austria	Yes (1996)	Yes (1996)	No
Belgium	No	Yes (1991)	No
Denmark	Yes (1993)	No	No
Finland	Yes (1994)	No	No
France	No	Yes (1986)	No
Germany	Yes (1979)	No	No
Greece	Yes (1982)	No	No
Iceland	Yes (1999)	No	No
Ireland	No	Yes (early 1990s)	Yes (See text for further information)
Italy	No	Yes (2000)	Yes (See text for further information)
Luxembourg	Yes (2000)	No	No
Netherlands	Only for food poisonings	Yes (1995)	Yes (See text for further information)
Norway	Yes (1979)	No	No
Portugal	No	No	Yes (See text for further information)
Spain	No	Yes (1989)	No
Sweden	Yes (1989)	No	No
Switzerland	Yes (1987)	No	No
United Kingdom	Only for food poisonings	Yes (2000)	Yes (See text for further information)

Table 3. Campylobacter notifying partners and site of central data collation within statutory (STAT) and sentinel surveillance (SENT) systems in European countries (n = 17), Survey I, 2000.

	Physicians		Laboratories			l health orities	Hospita	ls
	STAT	SENT	STAT	SENT	STAT	SENT	STAT	SENT
Austria	NPHI <sup>1</sup>			NRL <sup>1</sup>				
Belgium				NPHI				NPHI
Denmark			NRL					
Finland			NPHI					
France <sup>2</sup>				NRL				
Germany	NPHI <sup>3</sup>		NPHI <sup>3</sup>					
Greece			NPHI					
Iceland	NPHI		NPHI		NPHI			
Ireland				OTHER <sup>4</sup>		OTHER <sup>4</sup>		
Italy				NPHI				
Luxembourg	NPHI							
The Netherlands				NPHI				
Norway	NPHI		NPHI		NPHI			
Spain				NPHI				
Sweden	NPHI		NPHI					
Switzerland			NPHI					
United Kingdom				NPHI		NPHI		NPHI

<sup>1</sup> NPHI = National Public Health Institute, NRL = National Reference Laboratory <sup>2</sup> Hospital laboratories <sup>3</sup> Until the end of 2000 physicians notified but from 2001 only laboratories notify

4 Two separate regional laboratory surveillance systems, LSS and INFOSCAN. At present no systematic collation of data from these 2 systems.

Table 4. Frequency of notifications and forwarded form of data from local to the national level in European countries (n=16)	,
Survey I, 2000.	

	Continuously	Weekly	Monthly	Form of data
Statutory notification:				
Austria			Х	Aggregated
Denmark		Х		Aggregated
Finland		Х		Single cases
Germany		х		Aggregated form until end of 2000, single cases from 2001
Iceland	Х			Single cases
Luxembourg	Х			Single cases
Norway	Х			Single cases
Sweden	Х			Single cases
Switzerland	Х			Single cases
Sentinel surveillance:				
Austria	Х			Single cases
Belgium		Х		Single cases
France	Х			Single cases
Ireland		Х		Single cases
Italy	Х			Single cases
The Netherlands		Х		Aggregated
Spain		Х		Single cases
United Kingdom		Х	Х	Single cases

	Demograp	hical dat	а	Notifiable /	Notified disease	Clinical information		
	Name	Age	Sex	Verified diagnosis	Chronic carrier status	Symptoms	Hospitalisation	Outcome
Austria <sup>1</sup>	Х*	Х*	Х*	Х			Х	
Belgium	Х	х	х	Х				
Denmark	civil register no.	Х	х	Х				
Finland		Х	х	х				
France	initials	Х	х	Х		Х	Х	х
Germany <sup>2</sup>	x <sup>3</sup>	х	х	х		Х	Х	х
Iceland	Х	Х	х	Х				
Ireland	initials	х	х	х				
Italy	Х	Х	х			Х	Х	
Luxembourg	Х	Х	х	х				
Netherlands				Х				
Norway	Х	х	х	Х	Х	Х	Х	х
Spain		Х	х	Х				
Sweden	Х	х	х	Х				
Switzerland	initials	х	х	Х		Х	Х	
United Kingdom	Х	х	Х	Х		х	Х	х

### Table 5. Data submitted routinely in statutory or sentinel surveillance systems in European countries (n=16), Survey I, 2000

<sup>1</sup>Austria: has statutory and sentinel surveillance; data with \* are submitted by both systems, data without \* only by sentinel system <sup>2</sup>Germany: the variables listed will be available from 2001 <sup>3</sup>Only local level

#### Table 5 continued. Data submitted routinely in statutory or sentinel surveillance systems in European countries (n=16), Survey I, 2000

	Ep	oidemiologica	demiological information			Laboratory information			Sending institut	
	Risk factors	Source of infection	Relation to cases	Travel history	Name of laboratory	Method	Investigated material	Species	Name	Address
Austria <sup>1</sup>		х	х	х	Х	х	Х	Х	X*	Х*
Belgium					х		х			
Denmark				х	Х		х		х	х
Finland					х	culture only	х	х	х	х
France	х			х	Х		х	х	х	х
Germany <sup>2</sup>			х	х	x <sup>3</sup>	х	х	х	х	х
Iceland	х	х	х	х	Х				х	х
Ireland					Х		х	х	х	х
Italy	х	х	х	х	Х	х	Х	х	х	х
Luxembourg					Х					
Netherlands					Х		Х			
Norway	х	х	х	х	Х	х	х	х	х	х
Spain					Х	х	х	х		
Sweden		х	х	х	х	х	х		х	х
Switzerland					Х	х	х	х	х	х
United Kingdom	х	Х	Х	Х	х		х			х

<sup>1</sup>Austria: has statutory and sentinel surveillance; data with \* are submitted by both systems, data without \* only by sentinel system <sup>2</sup>Germany: the variables listed here will be available from 2001 <sup>3</sup>Only on local level

	Case definition	Since year	Description
Austria	Yes	1996	All patients with Campylobacter isolated from stool specimen or blood culture
Belgium	No		
Denmark	Yes	1993	Any <i>Campylobacter</i> species isolated from any site in one person in a 6-month period
Finland	Yes	1994	<i>Campylobacter</i> culture positive from any type of sample in one person in a 12- month period
France	No		
Germany	No (until 2000)	2001	Campylobacter culture positive from stool with or without clinical signs or a clinical case in a laboratory confirmed epidemic
Greece	Not reported		
Iceland	No		
Ireland	No		
Italy	Yes	Not reported	All patients with Campylobacter isolated from stool specimen or blood culture
Luxembourg	Yes	2000	Symptoms and culture positivity
The Netherlands	No		
Norway	Yes	1979	Laboratory finding of Campylobacter spp.
Spain	Yes	Not reported	A laboratory diagnosis of case isolates
Sweden	Yes	1989	A person from whom Campylobacter spp. has been isolated
Switzerland	Yes	1987	Culture of Campylobacter
United Kingdom	Yes	2000	Laboratory confirmed cases of Campylobacter infection

# Table 6. Case definitions for Campylobacter surveillance in European countries (n = 17), Survey I, 2000.

		Campyloba	cteriosis ca	ses / isolate	S		Notification rate / 100 000			
	1999	1998	1997	1996	1995	1999	1998	1997	1996	1995
Austria	3188	2454	1666	1131	unknown	39.4	30.3	21.4	14.5	unknown
Belgium	6514	6610	5617	4991	4879	63.6	65.0	54.9	49.8	48.7
Denmark	4164	3372	2666	2973	2601	78.0	63.6	50.0	57.6	50.0
England and Wales	54 994	58059	50177	43337	43876	104.9	110.7	96.5	83.3	84.6
Finland	3303	2851	2404	2629	2197	63.9	55.9	47.0	52.3	44.6
France			No data avai	lable			Ν	lo data availat	ole	
Germany	28882 <sup>2</sup>	33244 <sup>2</sup>	13095 <sup>3</sup>	10124 <sup>3</sup>	6600 <sup>4</sup>	65.2 <sup>2</sup>	75.0 <sup>2</sup>	70.0 <sup>3</sup>	54.0 <sup>3</sup>	37.0 <sup>4</sup>
Greece	306	136	N	o data availab	le	2.9	1.3	N	o data availat	ble
Iceland <sup>1</sup>	446	221	93	88	39	166.8	82.2	34.1	32.7	14.6
Ireland	2085	1318	943	646	644	57.5	36.1	26.0	20.1	17.8
Italy			No data avai	lable		No data available				
Luxembourg	171	176	152	136	141	40.7	41.9	36.2	32.4	33.6
Northern Ireland	858	775	778	652	557	50.8	46.3	46.5	39.5	33.8
Norway <sup>1</sup>	2027	1700	1178	1145	1046	45.6	38.5	27.3	26.5	24.2
Portugal			No data avai	lable			Ν	lo data availat	ole	
Scotland	5861	6375	5528	5098	4377	114.5	124.6	107.6	99.3	85.2
Spain	5191	4392	3755	3688	3237	13.2	11.2	9.6	9.4	8.2
Sweden	7137	6544	5306	5081	5580	80.5	74.0	60.0	57.4	63.2
Switzerland <sup>1</sup>	6709	5455	5955	5656	5044	93.7	76.5	83.7	79.6	71.2
The Netherlands	3135	3398	3646	3741	2871	32.3	33.8	37.3	39.2	29.8

#### Table 7. Reported Campylobacter infections in humans in European countries (n=18), 1995 to 1999 (The table is an adjusted version of the Table CA 1 from the report on trends and sources of zoonotic agents in the EU, 1998)

<sup>1</sup>Iceland. Norway and Switzerland are not members of the EU; Data on incidence cases/rates come from national surveillance programmes for infectious diseases <sup>2</sup>Data are related only to 11 Federal States; <sup>3</sup>Data are related only to 7 Federal States; <sup>4</sup>Data are related only to 6 Federal States

 Table 8. Existence of National Reference Laboratories (NRLs) and other laboratories performing reference tasks for human

 *Campylobacter* infections in European countries (n = 18), Survey I, 2000

	Existence of NRL for Campylobacter infections	Recommended NRL	Other laboratory performing reference tasks
Austria	Yes *		
Belgium	Yes *		
Denmark	Yes *		
Finland	No		
France	Yes *		
Germany	Yes *		In addition to NRL, a consulting laboratory for Campylobacter
Greece	Yes	Yes	
Iceland	Yes	Yes	
Ireland	No		
Italy	Yes		
Luxembourg	Yes *		
The Netherlands	No		
Norway	Yes	Yes	
Portugal	No		
Spain	No		
Sweden	No		SIIDC, Microbiologiska laboratorium, Salgrenska Sjukhuset
Switzerland	No		National Reference Laboratory for Foodborne Diseases, Institute of Veterinary Bacteriology, University of Bern
United Kingdom	Yes *		

\*Officially assigned

	Confirmation of results	New typing methods	New analytical methods	Develop proposals for standardisation	Conduct training courses	Routine primary diagnosis of specimen
Austria	Yes	No	No	Yes	No	Yes
Belgium	Yes	Yes	Yes	No	Yes	Yes
Denmark	Yes	Yes	Yes	Yes	Yes	Yes
France	Yes	Yes	Yes	Yes	Yes	Yes (only local specimen)
Germany	Yes	Yes (serotyping)	Yes	Yes	No	Yes
Greece	Yes	Yes	Yes	Yes	Yes	No
Iceland	Yes	No	No	Yes	Yes	Yes
Italy	Yes	No	No	No	Yes	No
Luxembourg	Yes	Yes	Yes	Yes	No	Yes
Sweden	Yes	Yes	No	No	No	No
Switzerland	No	Yes	Yes	No	No	No
Norway	Yes	Yes	Yes	Yes	No	No
United Kingdom	Yes	Yes	Yes	Yes	Yes	No

# Table 9. Tasks of the NRLs in European countries (n=13), Survey I, 2000

	Examine official specimen for monitoring	Coordinate methods for antibiotic resistance testing	Provide reference material for research and training	Coordinate application of typing methods	Provide reference material for laboratories
Austria	Yes	Yes	No	No	No
Belgium	Yes	No	No	No	No
Denmark	No	Yes	Yes	Yes	Yes
France	unknown	No	No	No	No
Germany	No	No	Yes	No	No
Greece	Yes	Yes	Yes	Yes	No
Iceland	Yes	No	No	No	No
Italy	No	No	No	No	No
Luxembourg	Yes	No	No	No	No
Norway	No	No	No	No	No
Sweden	No	No	No	No	No
Switzerland	No	No	No	Yes	No
United Kingdom	No	Yes	No	No	No

# Table 9 continued. Tasks of the NRLs in European countries (n=13), Survey I, 2000

	All isolates from the country	If No, who sends samples	When are isolates sent?
Austria	No	3 main laboratories in one region send isolates routinely (covering 1.2 Mio. population))	1, 2, 3
Belgium	No	Only strains from blood, CSF <sup>1</sup> , pus, stools only during outbreaks	1,3
Denmark	No	Local clinical hospital laboratories	1
France	No	From a network of hospital laboratories throughout the country	1, 2, 3
Germany	No	Institutes / official / private laboratories interested in specification of <i>Campylobacter</i> strains	1, 3
Greece	No	Hospitals with special interest (children's hospitals)	1, 4
Iceland	Yes		1, 2
Italy	No	Clinical microbiological laboratories	1, 4
Luxembourg	Yes		2
Norway	No	Some laboratories send all isolates, others only when they have a diagnostic problem	1, 4
Sweden	No	Other laboratories when special problems occur	1
Switzerland	No	Clinical microbiology, veterinary microbiology and food hygiene laboratories	1, 3
United Kingdom	No	Sentinel laboratories (sporadic infections) + any laboratory in the event of an outbreak	1, 2 (sentinels only), 3

# Table 10. Origin of human Campylobacter isolates received by NRLs in European countries (n=13), Survey I, 2000

<sup>1</sup>CSF: Cerebro-spinal fluid

#### Explanations of numbers 1-4 in the table:

- 1: When there is a diagnostical problem
- 2: Every isolate within a fixed time interval
- 3: Only when there is suspicion of an outbreak situation
- 4: At No regular time interval: ad hoc basis

	Medium	Incubation temperature		Incubation time
	-	37ºC	42°C	
Austria	mCCDA		Х	2 days
Belgium	Butzler's	Х	Х	3-5 days <sup>1</sup>
Denmark	mCCDA Blood agar (filtration)	Х		2 days
France	Karmali's Blood agar (filtration)	Х		2 days
Germany	mCCDA Skirrow's Abeyta-Hunt-Bark-agar (FDA) Columbia agar with 7% horse blood + Campy Supplement SR 84 (Oxoid)	X X		2 days 2 days
Greece	Skirrow's Blood agar (filtration)		X X	2 days 2 days
Iceland	mCCDA Skirrow's	Х	х	2 – 3 days 2 – 3 days
Italy	Karmali's	Х		2 days
Luxembourg	mCCDA	Х	Х	2 days
Norway	mCCDA <sup>2</sup>		Х	2 days
Sweden	Blood agar <sup>2</sup>	Х		
Switzerland	Butzler's Blood agar (filtration)	Х		2 days
United Kingdom	mCCDA	Х		2 days

### Table 11. Media and methods used for *Campylobacter* culturing by NRLs in European countries (n = 13), Survey I, 2000.

<sup>1</sup>5 days incubation for *C. upsaliensis* from paediatric patients <sup>2</sup>Only strains are cultured

Table 12. Methods for creating the microaerobic atmosphere for *Campylobacter* incubation by NRLs in European countries (n = 13), Survey I, 2000.

Microaerobic atmosphere	Country	Description
	Austria	
	France	Used sometimes
Commercial and neak	Germany	
Commercial gas pack	Greece	
	Iceland	
	Italy	
	Belgium	
	France	Anoxomat
Evenuation and rankagement system	Luxembourg	5% O <sub>2</sub> + 10% CO <sub>2</sub> + 85% N <sub>2</sub>
Evacuation and replacement system	Norway	5-10% O <sub>2</sub> + 10% CO <sub>2</sub>
	Sweden	5% O <sub>2</sub> + 10% CO <sub>2</sub> + 85% N <sub>2</sub>
	Switzerland	6% $O_2$ + 7% $CO_2$ + 7% $H_2$ + balance $N_2$
	Denmark	Microaerobic atmosphere + H <sub>2</sub>
Other	Italy	10% CO <sub>2</sub> incubator
	United Kingdom	Don Whiteley controlled atmosphere incubator

# Table 13. The use of filtration and enrichment for stool specimen processing for *Campylobacter* by NRLs in European countries (n = 13), Survey I, 2000.

	Filt	ration		Enrichment		
	Used	0.45 μ filter	0.60 μ filter	Filtr. time <sup>1</sup>	Used	Description
Austria	No				Never	
Belgium	Always	Х		2 x 30	Always	For isolation of Arcobacter
Denmark	In projects only		Х	30-45	Never	
France	Always		Х	10-15	Sometimes	I.e. in case of Guillain-Barré
Germany	Sometimes		Х		Sometimes	In outbreaks for food samples
Greece	Always		Х	60	Never	· · · ·
celand	No				Never	
taly	Always		Х	30-60	Never	
_uxembourg	Sometimes	Х			Sometimes	For food samples
Norway	Work only with pure cultures					•
Sweden	Work only with pure cultures					
Switzerland	Sometimes		Х	15	Never	
United Kingdom	Sometimes	Х		15	Sometimes	In outbreak investigations

<sup>1</sup>Filtration time in minutes

	C. jejuni	C. coli	C. lari	C. upsaliensis	Other spp.
Austria	Х	Х			
Belgium	Х	Х		Х	Arcobacter butzleri
Denmark	No growth	control			
France	No growth	control			
Germany	Х	Х			
Greece	Х	Х	Х		
Iceland	Х	Х	Х	Х	
Italy	Х	Х	Х		C. asylei, C. faecalis, C. hyointestinalis
Luxembourg	Х				
Norway	Х	Х	Х		
Sweden	Not reporte	ed			
Switzerland	Х			Х	H. pullorum
United Kingdom	Х	Х	Х		C. fetus

# Table 14. *Campylobacter* species used for growth control by NRLs in European countries (n = 13), Survey I, 2000.

	Medium	Tem	perature
		- 70ºC	Other
Austria	Cryobank	Х	
Belgium	Glycerol peptone medium	Х	
Denmark	10% glycerol	Х	
France	Glycerol + 20% peptone broth	Х	
Germany	Bolton's + 10% calf serum + 10% glycerol	Х	- 80°C
Greece	Trypticase soy broth + 20% glycerine		- 80°C and – 160°C
Iceland	Trypticase soy broth + glycerol + distilled water	Х	- 85°C
Italy	Blood, microbank		- 80°C
Luxembourg	Sheep blood + lyophilisation	X (sheep blood)	room temperature for lyophilisation
Norway	TSB + 10% glycerol + 10% horse serum	Х	
Sweden		Not reported	
Switzerland	TSB + 1.2% saccharose	Х	
United Kingdom	Microbank beads + 10% glycerol		- 80°C

# Table 15. Storage of Campylobacter isolates in NRLs in European countries (n=13), Survey I, 2000

	Oxidase	Catalase	Blood Agar	Hippurate hydrolysis	Indoxyl acetate hydrolysis	Direct microscopy	API Campy <sup>1</sup>	Urease	Nitrate reduction	H₂S / Pb- acetate
Austria	Х	Х	Х	Х	Х		Х			
Belgium	Х	Х	Х	Х	Х			Х	Х	Х
Denmark <sup>2</sup>						Х				
France	Х	Х				Х	Х			Х
Germany	Х	Х	Х	Х	Х	Х			Х	
Greece	Х	Х	Х	Х			Х			
Iceland	Х	Х	Х	Х	Х	Х	Х			
Italy	Х	Х	Х	Х		Х		Х	Х	Х
Luxembourg	Х	Х		Х		Х	Х	Х	Х	
Norway	Х	Х	Х	Х	Х	Х	Х	Х		Х
Sweden				Only g	enotyping					
Switzerland	Х	Х	Х		Х			Х	Х	Х
United Kingdom	Х	Х	Х	Х	Х			Х		
Total	11	11	9	9	8	7	6	6	5	5

# Table 16. Phenotypic identification of *Campylobacter spp.* in NRLs in European countries (n=13), Survey I, 2000

<sup>1</sup>Commercial test kit <sup>2</sup>Species-specific PCR in projects

	Müller- Hinton Broth 1.5% NaCl	Müller- Hinton Broth 1% Glycine	MacConkey agar	H₂S / Cysteine (Pb-acetate)	Nitrite reduction	Trypticase Soy Broth (TSB)	Müller-Hinton Broth	TSI, TSI+DNAse
Austria								
Belgium								
Denmark								
France								Х
Germany	Х	Х		Х				
Greece								
Iceland							Х	
Italy								
Luxembourg					Х			
Norway	Х	Х	Х	Х		Х		
Sweden			Only ge	enotyping				
Switzerland			Х					
United Kingdom	Х							
Total	3	2	2	2	1	1	1	1

Table 16 continued. Phenotypic identification of *Campylobacter spp.* in NRLs in European countries (n=13), Survey I, 2000

	Antimicrobial resistance pattern	Enzyme profile auxotyping	Total fatty acid gas chromatography
Austria	Х		
Belgium	Х		
Denmark	Х		
France	Х	Х	
Greece	Х		
Italy	Х		
Norway	Х		
United Kingdom	Х		Х

Table 17. Other reported methods for phenotyping by NRLs in European countries (n = 8), Survey I, 2000.

	Genom	Genomic DNA profiling			PCR-based profiling			
	Frequent cutting enzymes	PFGE	Ribotyping	RFLP	RAPD	AFLP	MLST	
Austria								
Belgium								
Denmark		Х	X <sup>1</sup>		Х			
France					Х	Х		
Germany		Х			Х			
Greece	Х	Х						
Iceland		Х						
Italy								
Luxembourg		Х	Х	Х				
Norway		Х		Х		Х		
Sweden			Х					
Switzerland		Х	Х	Х			Х	
United Kingdom		Х	Х	Х		Х	Х	
TOTAL	1	8	5	4	3	3	2	

Table 18. Genotyping methods in the NRLs in European countries (n = 13), Survey I, 2000.

<sup>1</sup>Riboprinting

# Table 19. Methods for *Campylobacter* susceptibility testing in NRLs in European countries (n = 13), Survey I, 2000

		MIC determinations				
	Agar diffusion	Agar dilution	E-test			
Austria	Routinely		When nalidixic resistance; ciprofloxacin sensitivity			
Belgium	Routinely		Routinely for macrolides and quinolones; at intervals for MIC testing			
Denmark	Routinely		To confirm unclear reactions & for quinolone resistant strains			
France	Routinely					
Germany	Routinely					
Greece		MIC testing: when 30-40 strains sent for testing at the laboratory at same the time	Routinely			
Iceland	For cephalotin and nalidixic acid <sup>1</sup>		For ciprofloxacin and erythromycin			
Italy	Routinely		To confirm unclear reactions			
Luxembourg			Random tests at intervals			
Norway						
Sweden	Not reported					
Switzerland	Routinely		For ciprofloxacin and erythromycin to confirm resistant strains			
United Kingdom		Routinely	For ciprofloxacin and erythromycin to confirm resistant strains			

<sup>1</sup>For species differentiation

# Table 20. Antimicrobial agents that were tested for *Campylobacter* susceptibility in the NRLs in European countries (n = 13), Survey I, 2000.

	Antimicrobial substances									
-	Nalidixic acid	Cephalotin	Erythromycin	Ciprofloxacin	Tetracyclin	Gentamycin	Ampicillin	Penicillin	Chloramphenicol	
Austria	Х		Х	Х	Х	Х	Х			
Belgium	Х	Х	Х	Х						
Denmark	Х		Х							
France	Х	Х	Х	Х	Х	Х	Х			
Germany	Х	Х						Х		
Greece	Х	Х	Х	Х	Х	Х	Х			
Iceland	Х	Х	Х	Х						
Italy	Х	Х	Х	Х	Х	Х	Х	Х		
Luxembourg	Х		Х	Х						
Norway	Х	Х	Х	Х	X <sup>1</sup>	Х				
Sweden					Not reported	b				
Switzerland	Х	Х	Х		Х					
United Kingdom	Х		Х	Х	Х	Х	Х		Х	
TOTAL	12	8	11	9	7	6	5	2	1	

<sup>1</sup>Doxycyclin

	Internal C	Internal QA		External QA		o adopt QA	Accreditation		
Austria		No		No	Yes			No	
Belgium		No		No	Yes			No	
Denmark	Yes		Yes					No <sup>1</sup>	
France		No		No		No		No	
Germany	Yes		Yes				Yes <sup>2</sup>		
Greece		No		No	Yes			No	
Iceland	Yes		Yes					No	
Italy		No		No	Yes			No	
Luxembourg	Yes			No	Yes			No	
Norway		No	Yes					No	
Sweden		No		No		No		No	
Switzerland	Yes			No		No	Yes <sup>2</sup>		
United Kingdom	Yes			No	Yes		Yes <sup>3</sup>		
TOTAL	6	7	4	9	6	3	3	10	

Table 21. Quality assurance (QA) and accreditation of *Campylobacter* diagnostics in NRLs in European countries (n = 13), 2000.

<sup>1</sup>In process

<sup>2</sup>According to EN 45001/ISO Guide 25 or ISO 17025 <sup>3</sup>Clinical Pathology Accreditation

		Non-responders								Responders								
	Hospital- based		I	PHL		Private		Other A		•	lospital- based		PHL		Private		Other	
	n	%	n	%	n	%	n	%	n	n	%	n	%	n	%	n	%	n
Austria	5	36	0	0	8	57	1	7	14	30	68	5	11	8	18	1	2	44
Denmark	4	100	0	0	0	0	0	0	4	6	86	1	14	0	0	0	0	7
England & Wales	5	17	12	41	0	0	12 <sup>1</sup>	41	29	4	67	2	33	0	0	0	0	6
Finland	2	50	0	0	2	50	0	0	4	15	71	1	5	4	19	1	5	21
France	37	15	0	0	218	85	0	0	255	63	26	0	0	182	74	0	0	245
Germany	n.a	a <sup>2</sup>	n.a	I	n.a		n.a	à	980	320	71	11	2	95	21	24	5	450
Greece	9 <sup>3</sup>	100	0	0	0	0	0	0	9	6	100	0	0	0	0	0	0	6
Ireland	4	100	0	0	0	0	0	0	4	46	94	1	2	1	2	1	2	49
Italy	44	73	12	20	2	3	2	3	60	110	83	10	8	6	5	6	5	132
Portugal	48	48	0	0	53	52	0	0	101	18	62	0	0	11	38	0	0	29
Scotland	13	100	0	0	0	0	0	0	13	23	92	0	0	1	4	1	4	25
TOTAL									1473	641	63	31	3	308	30	34	3	1014

#### Table 22. Characteristics of non-responding and responding Campylobacter laboratories in 10 European countries, Survey II, 2000

<sup>1</sup>National Health Service <sup>2</sup>Data not available <sup>3</sup>All non-responders don't perform *Campylobacter* diagnostics routinely

	<i>Campylobacter</i> laboratories		Hospital-based laboratory		c health oratory		Private laboratory		
	n	n	%	n	%	n	%	n	%
Austria	41	27	66	5	12	8	20	1 <sup>1</sup>	2
Denmark	7	6	86	1	14	0	0	0	0
England & Wales	6	4	67	2	33	0	0	0	0
Finland	21	15	71	1	5	4	19	1 <sup>1</sup>	5
France	217	59	27	0	0	157	72	1 <sup>2</sup>	0
Germany	138	51	37	10	7	71	51	6 <sup>3</sup>	4
Greece	6	6	100	0	0	0	0	0	0
Ireland	35	33	94	1	3	1	3	0	0
Italy	115	99	86	4	3	9	8	3 <sup>4</sup>	3
Portugal	13	10	77	0	0	3	23	0	0
Scotland	23	22	96	0	0	1	4	0	0
TOTAL	622	331	53	25	4	254	41	12	2

Table 23. Characteristics of primary Campylobacter laboratories in 10 European countries, Survey II, 2000

<sup>1</sup>University laboratory <sup>2</sup>Municipal laboratory <sup>3</sup>Five university laboratories and one army laboratory <sup>4</sup>One university and two other type of laboratories

	CampylobacterPatients fromlaboratoriesmajor hospitals			ents from are hospitals	Outp	oatients	Other patients		
	n	n	%	n	%	n	%	n	%
Austria	41	4	10	32	78	26	63	3	7
Denmark	7	1	14	6	86	7	100	0	0
England & Wales	6	6	100	3	50	2	33	0	0
Finland	21	12	57	12	57	5	24	0	0
France	217	82	38	51	24	168	77	13	6
Germany	138	45	33	76	55	79	57	23	17
Greece	6	3	50	2	33	2	33	1	17
Ireland	35	12	34	21	60	4	11	0	0
Italy <sup>a</sup>	115	54	47	34	30	78	68	19	17
Portugal	13	7	54	4	31	2	15	0	0
Scotland	23	12	52	12	52	6	26	5	22
TOTAL	622	238	38	253	41	379	61	64	10

#### Table 24. Patient groups served by *Campylobacter* laboratories in 10 European countries, Survey II, 2000

## Table 25. Demographic data transmitted to Campylobacter laboratories with sample submission in 10 European countries, Survey II, 2000

	<i>Campylobacter</i> laboratories	А	ge	ç	Sex	Resid	Residence		
	n	n	%	n	%	n	%		
Austria	41	41	100	40	98	21	51		
Denmark	7	7	100	7	100	1	14		
England & Wales	6	6	100	5	83	3	50		
Finland	21	21	100	21	100	17	81		
France	217	177	82	194	89	133	61		
Germany	138	134	97	130	94	108	78		
Greece	6	5	83	6	100	4	67		
Ireland	35	33	94	33	94	29	83		
Italy	115	99	86	105	91	77	67		
Portugal	13	12	92	13	100	12	92		
Scotland	23	23	100	23	100	20	87		
TOTAL	622	558	90	577	93	425	68		

	<i>Campylobacter</i> laboratories		specimen ection		ory of rhoea	Sympto	m onset	Hospitalisation	
	n	n	%	n	%	n	%	n	%
Austria	41	34	83	32	78	3	7	14	34
Denmark	7	6	86	5	71	2	29	3	43
England & Wales	6	6	100	6	100	2	33	2	33
Finland	21	21	100	10	48	5	24	6	29
France	217	186	86	88	41	12	6	64	29
Germany	138	106	77	64	46	10	7	24	17
Greece	6	6	100	6	100	4	67	4	67
Ireland	35	27	77	16	46	0	0	2	6
Italy	115	87	76	23	20	17	15	76	66
Portugal	13	13	100	9	69	3	23	5	38
Scotland	23	23	100	20	87	10	43	11	48
TOTAL	622	515	83	279	45	68	11	211	34

# Table 26. Data of clinical history transmitted to Campylobacter laboratories with sample submission in 10 European countries,Survey II, 2000

	<i>Campylobacter</i> laboratories	Part of an outbreak		Tra	vel
	n	n	%	n	%
Austria	41	12	29	12	29
Denmark	7	2	29	2	29
England & Wales	6	3	50	5	83
Finland	21	12	57	19	90
France	217	10	5	28	13
Germany	138	29	21	37	27
Greece	6	3	50	3	50
Ireland	35	13	37	12	34
Italy	115	30	26	29	25
Portugal	13	4	31	2	15
Scotland	23	12	52	18	78
TOTAL	622	130	21	167	27

## Table 27. Other data transmitted to Campylobacter laboratories with sample submission in 10 European countries,Survey II, 2000

	<i>Campylobacter</i> laboratories	000	1000 -	10 000	> 10 000	Total replied		
	n	n	%	n	%	n %	n	%
Austria	41	3	8	27	73	7 19	37	90
Denmark	7	0	0	4	57	3 43	7	100
<b>England &amp; Wales</b>	6	0	0	3	50	3 50	6	100
Finland	21	1	5	16	76	4 19	21	100
France	217	181	89	23	11	0 <i>0</i>	204	94
Germany	138	9	7	68	53	52 40	129	93
Greece	6	2	33	4	67	0 0	6	100
Ireland	35	9	36	16	64	0 <i>0</i>	25	71
Italy	115	25	23	82	75	2 2	109	95
Portugal	13	11	85	2	15	0 0	13	100
Scotland	23	4	19	14	67	3 14	21	91
TOTAL	622	245	42	259	45	74 13	578	93

Table 28. The size of Campylobacter laboratories by total number of stool samples in 10 European countries in 1999,Survey II, 2000

	<i>Campylobacter</i> laboratories	Sum of stool samples						isolat	f positive es / 100 gations <sup>1</sup>
	n	N <sup>2</sup>	N <sup>2</sup> x 1000		N x 1000		sum	Ν	mean
Austria	41	37	396	35	173	34	6199	16	2.8
Denmark	7	7	122	6	118	7	4871	4	4.8
<b>England &amp; Wales</b>	6	6	54	5	47	6	2820	2	5.4
Finland	21	21	134	21	62	21	3664	13	6.2
France	217	204	100	174	42	145	1405	72	3.4
Germany	138	129	1963	125	822	122	24464	40	2.2
Greece	6	6	12	6	10	6	364	4	4.2
Ireland	35	25	58	8	31	18	1193	2	3.2
Italy	115	109	310	111	140	96	2629	47	2.8
Portugal	13	13	7	13	3	11	102	9	5.6
Scotland	23	21	106	21	105	18	5057	7	4.7
TOTAL	622	578	3262	525	1553	484	52768	216	3.4

Table 29. Total sums of stool samples, *Campylobacter* investigations and positive results and mean isolation rates in 10 European countries in 1999, Survey II, 2000.

<sup>1</sup>Based on replies with precise data for investigations and results <sup>2</sup>No of laboratories

	<i>Campylobacter</i> laboratories	ries No findings 1		1 -	<u>1 - 99</u> <u>100 - 1</u>					Total replied	
	n	n	%	n	%	n	%	n	%	n	%
Austria	41	1	3	18	51	16	46	0	0	35	85
Denmark	7	0	0	0	0	6	86	1	14	7	100
England & Wales	6	0	0	1	17	4	67	1	17	6	100
Finland	21	0	0	9	43	12	57	0	0	21	100
France	217	46	24	144	75	1	1	0	0	191	88
Germany	138	2	2	70	56	46	37	6	5	124	90
Greece	6	0	0	4	67	2	33	0	0	6	100
Ireland	35	0	0	14	78	4	22	0	0	18	51
Italy	115	7	7	92	89	4	4	0	0	103	90
Portugal	13	2	15	11	85	0	0	0	0	13	100
Scotland	23	0	0	2	11	16	89	0	0	18	78
TOTAL	622	58	11	365	67	111	20	8	1	542	87

### Table 30. *Campylobacter* findings by primary laboratories in 10 European countries in 1999, Survey II, 2000

	<i>Campylobacter</i> laboratories	Rout	tinely	•			oreaks	Blood/mucus in sample		Ot	Other	
	n	n	%	n	%	n	%	n	%	n	%	
Austria	41	30	73	6	15	3	7	1	2	6	15	
Denmark	7	7	100									
England & Wales	6	5	83							1	17	
Finland	21	3	14	18	86	8	38	2	10			
France	217	79	36	128	59	48	22	132	61	105	48	
Germany	138	36	26	73	53	43	31	40	29	62	45	
Greece	6	5	83	1	17							
Ireland	35	33	94					1	3	2	6	
Italy	115	55	48	47	41	15	13	33	29	28	24	
Portugal	13	8	62	4	31	2	15	3	23	1	8	
Scotland	23	22	96	3	13	3	13	3	13	1	4	
TOTAL	622	283	45	280	45	122	20	215	35	206	33	

Table 31. Specifications for *Campylobacter* investigation in primary laboratories in 10 European countries, Survey II, 2000

	Campylobacter Method laboratories published		Not pul	blished	Don't k	now	Total ı	Total replied	
	n	n	%	n	%	n	%	n	%
Austria	41	30	75	2	5	8	20	40	98
Denmark	7	3	43	2	29	2	29	7	100
England & Wales	6	5	100	0	0	0	0	5	83
Finland	21	10	48	7	33	4	19	21	100
France	217	82	42	35	18	77	40	194	89
Germany	138	102	78	11	8	17	13	130	94
Greece	6	5	100	0	0	0	0	5	83
Ireland	35	17	50	7	21	10	29	34	97
Italy	115	64	57	33	29	15	13	112	97
Portugal	13	3	23	9	69	1	8	13	100
Scotland	23	9	41	6	27	7	32	22	96
TOTAL	622	330	57	112	19	141	24	583	94

Table 32. Application of published guidelines in *Campylobacter* laboratories in 10 European countries, Survey II, 2000

	Campylobacter	I	Direct microsco	ору			Filtration		
	laboratories	Always	Sometimes	То	tal	Always	Sometimes	Tot	tal
	n	n	n	n	%	n	n	n	%
Austria	41	0	3	3	7	0	0	0	0
Denmark	7	0	0	0	0	0	1	1	14
England & Wales	6	0	1	1	17	0	0	0	0
Finland	21	1	0	1	5	0	0	0	0
France	217	160	30	190	88	3	3	6	3
Germany	138	4	8	12	9	3	1	4	3
Greece	6	3	1	4	67	0	0	0	0
Ireland	35	0	0	0	0	0	0	0	0
Italy	115	15	25	40	35	12	7	19	17
Portugal	13	5	0	5	38	0	0	0	0
Scotland	23	1	0	1	4	0	0	0	0
TOTAL	622	189	68	257	41	18	12	30	5

## Table 33. Use of direct stool microscopy and filtration for Campylobacter investigations in 10 European countries,Survey II, 2000

	Campylobacter	Sedime	entation		Enrichmen	t	
	laboratories			Always	Sometimes	Тс	otal
	n	n	%	n	n	n	%
Austria	41	0	0	0	0	0	0
Denmark	7	1	14	0	1	1	14
England & Wales	6	0	0	0	0	0	0
Finland	21	0	0	0	0	0	0
France	217	2	1	51	3	54	25
Germany	138	2	1	5	2	7	5
Greece	6	0	0	1	0	1	17
Ireland	35	0	0	1	0	1	3
Italy	115	10	9	10	4	14	12
Portugal	13	0	0	1	0	1	8
Scotland	23	1	4	1	0	1	4
TOTAL	622	16	3	70	11	80	13

Table 34. Use of sedimentation or enrichment for *Campylobacter* investigations in 10 European countries, Survey II, 2000

	<i>Campylobacter</i> laboratories	Karmali / CSM	mCCDA	Campy Bap	Skirrow	Butzler	Blaser- Wang	Preston	САТ	Campylosel
	n	n	n	n	n	n	n	n	n	n
Austria	41	3	4	7	2	1	0	5	1	15
Denmark	7	0	7	0	0	0	0	0	0	0
England & Wales	6	1	2	0	0	0	0	2	1	0
Finland	21	2	18	1	0	0	0	0	0	0
France	217	22	0	2	2	7	0	0	0	181
Germany	138	22	24	20	21	23	7	8	2	10
Greece	6	0	0	0	5	0	1	0	0	0
Ireland	35	0	19	0	3	0	0	11	0	0
Italy	115	26	14	20	12	4	10	13	5	10
Portugal	13	0	0	5	0	0	0	0	2	6
Scotland	23	1	7	0	9	1	0	6	1	0
TOTAL	622	77 (12%)	95 (15%)	55 (9%)	54 (9%)	36 (6%)	18 (3%)	45 (7%)	12 (2%)	222 (36%)

### Table 35. Primary media used for culturing *Campylobacter* in 10 European countries, Survey II, 2000

Incubation time h/	Karma	li / CSM	mCC	CDA	Camp	у Вар	Skir	row	But	zler	Blaser-	Wang	Pres	ton	С	AT	Camp	ylose	TOTAL
temperature °C	37º	42º	37º	42º	37º	42º	37º	42º	37º	42º	37º	42º	37º	42º	37º	42º	37º	42º	no of labs
24 h						1					1						3	1	6
42 h											1						1		2
44-48 h	18	29	26	43	9	31	10	31	9	20	4	7	3	30	6	2	96	49	423
62 h				1															1
72 h	5	6	8	4	1	2	1	4	2	1			1	3		2	17	12	69
78 h																		1	1
96 h				2				1									2		5
106 h																		1	1
120 h	1		1	1													7	2	12
144 h		1																	1
168 h												1						1	2
TOTAL no of labs	24	36	35	51	10	34	11	36	11	21	6	8	4	33	6	4	126	67	523

Table 36. Primary media, incubation time and incubation temperature (°C) in *Campylobacter* laboratories in 10 European countries, Survey II, 2000

	<i>Campylobacter</i> laboratories	Comm gas j		Cand	lle jar	ar	uation nd ement	Otl	ner	Total	replied
	n	n	%	n	%	n	%	n	%	n	%
Austria	41	35	85	1	2	0	0	5	12	41	100
Denmark	7	3	43	0	0	2	29	2	29	7	100
England & Wales	6	5	83	0	0	1	17	0	0	6	100
Finland	21	15	71	0	0	5	24	1	5	21	100
France	217	189	87	7	3	2	1	13	6	211	97
Germany	138	104	75	3	2	23	17	7	5	137	99
Greece	6	6	100	0	0	0	0	0	0	6	100
Ireland	35	34	97	0	0	0	0	1	3	35	100
Italy	115	109	95	0	0	3	3	3	3	115	100
Portugal	13	12	92	0	0	1	8	0	0	13	100
Scotland	23	20	87	0	0	2	9	1	4	23	100
TOTAL	622	532	86	11	2	39	6	33	5	615	99

## Table 37. Microaerobic atmosphere for Campylobacter culturing in primary laboratories in 10 European countries, Survey II, 2000

	<i>Campylobacter</i> laboratories	One samp	le / plate	> 1 sam plat	-	Total	replied
	n	n	%	n	%	n	%
Austria	41	11	27	30	73	41	100
Denmark	7	7	100	0	0	7	100
England & Wales	6	3	50	3	50	6	100
Finland	21	21	100	0	0	21	100
France	217	202	93	12	6	214	99
Germany	138	95	69	41	30	136	99
Greece	6	3	50	3	50	6	100
Ireland	35	32	91	3	9	35	100
Italy	115	103	90	11	10	114	99
Portugal	13	10	77	3	23	13	100
Scotland	23	21	91	2	9	23	100
TOTAL	622	508	82	108	17	616	99

### Table 38. Culturing practices in Campylobacter laboratories in 10 European countries, Survey II, 2000

	-	pylobact oratories		Micro	scopy	Oxi	dase	Cata	alase		itex ination	Otl	her
-	n	Conf <sup>1</sup>	%	n	%	n	%	n	%	n	%	n	%
Austria	41	39	95	38	97	34	87	22	54	9	22	4	10
Denmark	7	7	100	7	100	6	86	6	86	0	0	0	0
England & Wales	6	6	100	6	100	4	67	0	0	0	0	2	33
Finland	21	21	100	21	100	21	100	8	38	1	5	5	24
France	217	187	86	184	98	162	87	147	68	0	0	29	13
Germany	138	136	99	132	97	129	95	102	74	12	9	32	23
Greece	6	6	100	6	100	6	100	6	100	1	17	1	17
Ireland	35	35	100	35	100	32	91	10	29	9	26	6	17
Italy	115	111	97	106	95	97	87	84	73	36	31	20	17
Portugal	13	13	100	12	92	12	92	11	85	1	8	4	31
Scotland	23	23	100	23	100	22	96	5	22	0	0	1	4
TOTAL	622	584	94	570	98	525	90	401	64	69	11	104	17

#### Table 39. Confirmation methods in *Campylobacter* laboratories in 10 European countries, Survey II, 2000

<sup>1</sup>Confirm the isolates

	Campylobacter laboratories			urate olysis	Nitr redue		Indo acet			₂S uction		lixic acid eptibility	Ot	her	
	n	Det <sup>1</sup>	%	n	%	n	%	 n	%	n	%	n	%	n	%
Austria	41	17	41	11	65	2	12	3	18	2	12	8	47	11	65
Denmark	7	1	14	0	0	0	0	0	0	0	0	0	0	1	100
England & Wales	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Finland	21	20	95	20	100	4	20	1	5	1	5	9	45	7	35
France	217	111	51	47	42	15	14	2	2	13	12	70	63	63	57
Germany	138	112	81	81	72	15	13	14	13	9	8	71	63	53	47
Greece	6	5	83	4	80	3	60	1	20	3	60	5	100	1	20
Ireland	35	20	57	11	55	0	0	3	15	0	0	11	55	6	30
Italy	115	69	60	34	49	24	35	11	16	23	33	42	61	51	74
Portugal	13	9	69	4	44	1	11	0	0	1	11	4	44	7	78
Scotland	23	3	13	3	100	1	33	1	33	2	67	1	33	1	33
TOTAL	622	367	59	215	59	65	18	36	10	54	15	221	60	201	55

Table 40. Tests applied to identify the Campylobacter species in primary laboratories in 10 European countries, Survey II, 2000

<sup>1</sup>Determine the isolates to the species level

	<i>Campylobacter</i> laboratories	Rout	inely	Isolate: outbr		Some	times	N	lo	Total r	eplied
	n	n	%	n	%	n	%	n	%	n	%
Austria	41	3	7	2	5	4	10	32	78	41	100
Denmark	7	0	0	0	0	1	14	6	86	7	100
<b>England &amp; Wales</b>	6	2	33	0	0	1	17	3	50	6	100
Finland	21	3	14	2	10	4	19	12	57	21	100
France	217	30	14	20	10	151	73	5	2	206	95
Germany	138	20	14	2	1	22	16	94	68	138	100
Greece	6	5	83	0	0	1	17	0	0	6	100
Ireland	35	7	20	0	0	2	6	25	71	34	97
Italy	115	15	13	2	2	17	15	80	70	114	99
Portugal	13	1	8	0	0	2	15	10	77	13	100
Scotland	23	4	17	0	0	0	0	19	83	23	100
TOTAL	622	90	15	28	5	205	34	286	47	609	<b>98</b>

Table 41. Storage practices for *Campylobacter* isolates in primary laboratories in 10 European countries, Survey II, 2000

	<i>Campylobacter</i> laboratories	Storag repo		Da	ays	Мо	nths	Y	ears
	n	n	%	n	%	n	%	n	%
Austria	41	9	22	1	11	3	33	5	56
Denmark	7	1	14	0	0	0	0	1	100
England & Wales	6	3	50	0	0	1	33	2	67
Finland	21	9	43	0	0	2	22	7	78
France	217	46	21	11	24	10	22	25	54
Germany	138	39	28	9	23	11	28	19	49
Greece	6	5	83	0	0	0	0	5	100
Ireland	35	8	23	3	38	0	0	5	63
Italy	115	29	25	4	14	6	21	19	66
Portugal	13	3	23	0	0	2	67	1	33
Scotland	23	3	13	1	33	1	33	1	33
TOTAL	622	155	25	29	19	36	23	90	58

Table 42. Storage times for *Campylobacter* isolates in primary laboratories in 10 European countries (n = 155), Survey II, 2000

<sup>1</sup>Practices (always, in outbreaks, occasionally) grouped together

	<i>Campylobacter</i> laboratories	Total	replied	Alv	ways	Someti	nes	N	ю
	n	n	%	n	%	n	%	n	%
Austria	41	41	100	24	59	6	15	11	27
Denmark	7	6	86	2	33	0	0	4	67
England & Wales	6	6	100	3	50	1	17	2	33
Finland	21	21	100	11	52	3	14	7	33
France	217	210	97	94	45	9	4	107	51
Germany	138	138	100	60	43	19	14	59	43
Greece	6	6	100	6	100	0	0	0	0
Ireland	35	33	94	13	39	4	12	16	48
Italy	115	114	99	59	52	14	12	41	36
Portugal	13	13	100	1	8	0	0	12	92
Scotland	23	23	100	10	43	3	13	10	43
TOTAL	622	611	98	283	46	59	10	269	44

Table 43. Antimicrobial susceptibility testing in *Campylobacter* laboratories in 10 European countries (n = 611), Survey II, 2000

	<i>Campylobacter</i> laboratories	perfo susce	atories orming ptibility ting <sup>1</sup>	Agar di	ffusion	E te	est	Agar di	ilution	Oth	her
	n	n	%	n	%	n	%	n	%	n	%
Austria	41	30	73	26	87	5	17	0	0	0	0
Denmark	7	2	29	1	50	0	0	0	0	0	0
England & Wales	6	4	67	4	100	0	0	0	0	0	0
Finland	21	14	67	14	100	1	7	0	0	0	0
France	217	103	47	75	73	0	0	4	4	16	16
Germany	138	79	57	76	96	4	5	0	0	1	1
Greece	6	6	100	6	100	1	17	0	0	0	0
Ireland	35	17	49	17	100	1	6	0	0	0	0
Italy	115	73	63	63	86	6	8	0	0	6	8

1 100

Table 44. Antimicrobial susceptibility testing methods in *Campylobacter* laboratories in 10 European countries (n = 342), Survey II, 2000

<sup>1</sup>Practices (always and occasionally) grouped

Portugal

Scotland

TOTAL

Table 45. Antimicrobial agents for Campylobacter susceptibility testing in primary laboratories in 10 European	countries
(n = 342), Survey II, 2000	

			Ciprofloxacin	Gentamucio	Gentaniyoni	Tetracvclin		Nalidivic acid		Eruthromucin	LI yuu omyom		Cephalotti	anii o i no d		Ambicillin	-	Chloramphenicol	
	n	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Austria	30	29	97	4	13	18	60	9	30	28	93	5	17	1	3	5	17	0	0
Denmark	2	2	100	0	0	0	0	1	50	2	100	0	0	0	0	1	50	0	0
England & Wales	4	3	75	0	0	0	0	0	0	4	100	0	0	0	0	0	0	0	0
Finland	14	13	93	1	7,1	4	29	6	43	13	93	5	36	0	0	2	14	1	7
France	103	80	78	86	83	77	75	73	71	91	88	85	83	27	26	90	87	43	42
Germany	79	71	90	33	42	54	68	40	51	73	92	45	57	19	24	44	56	12	15
Greece	6	4	67	4	67	3	50	6	100	6	100	5	83	0	0	5	83	3	50
Ireland	17	12	71	5	29	3	18	10	59	16	94	0	0	0	0	3	18	1	6
Italy	73	60	82	55	75	56	77	39	53	68	93	43	59	14	19	47	64	51	70
Portugal	1	0	0	0	0	0	0	0	0	0	0	1	100	0	0	1	100	0	0
Scotland	13	10	77	2	15	4	31	3	23	12	92	2	15	1	8	4	31	1	8
TOTAL	342	284	83	190	56	219	64	187	55	313	92	191	56	62	18	202	59	112	33

	Campylobacter				Always	to		(	Occasional	ly to	
	laboratories	Total rep	olied	NRL	Other	Т	otal	NRL	Other	т	otal
	n	n	%	n	n	n	%	n	n	n	%
Austria	41	41	100	5	0	5	12	1	2	3	7
Denmark	7	7	100	0	0	0	0	1	1	2	29
England & Wales	6	6	100	5	0	5	83	1	0	1	17
Finland	21	21	100	0	0	0	0	5	2	7	33
France	217	213	98	6	21	27	13	19	24	43	20
Germany	138	137	99	2	2	4	3	14	4	18	13
Greece	6	6	100	2	0	2	33	0	1	1	17
Ireland	35	34	97	0	2	2	6	2	8	10	29
Italy	115	114	99	11	10	21	18	5	8	13	11
Portugal	13	13	100	0	0	0	0	0	0	0	0
Scotland	23	23	100	0	0	0	0	3	0	3	13
TOTAL	622	615	99	31	35	66	11	51	50	101	16

Table 46. Practices in sending Campylobacter isolates for further characterisation in 10 European countries (n = 615), Survey II,2000

	<i>Campylobacter</i> laboratories	Tot	al	ŀ	Always	or occa	sional	ly to <sup>1</sup>			Campylobacter			r	
		replied		LHA		I	NSC		NRL		her	is not reportable		No	
	n	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Austria	41	41	100	17	41	0	0	2	5	1 <sup>2</sup>	2	0	0	21	51
Denmark	7	6	86	0	0	5 <sup>3</sup>	83	5 <sup>3</sup>	83			0	0	0	0
England & Wales	6	6	100	6	100	5	83	4	67			0	0	0	0
Finland	21	21	100	3	14	19	90	0	0			0	0	0	0
France	217	190	88	2	1	0	0	4	2	3 <sup>4</sup>	2	177	93	4	2
Germany	138	138	100	126	91	0	0	0	0			0	0	12	9
Greece	6	6	100	1	17	4	67	0	0			0	0	1	17
Ireland	35	33	94	19	58	1	3	0	0	<b>3</b> <sup>5</sup>	9	0	0	13	39
Italy	115	112	97	8	7	8	7	9	8	2 <sup>6</sup>	2	3	3	86	77
Portugal	13	13	100	0	0	0	0	0	0			1	8	12	92
Scotland	23	23	100	22	96	17	74	0	0	1 <sup>7</sup>		0	0	0	0
TOTAL	622	589	95	204	35	59	10	24	4	10	2	181	31	149	25

#### Table 47. Reporting routines of *Campylobacter* positive samples from primary laboratories in European countries (n = 589), Survey II, 2000

<sup>1</sup>Some laboratories reported to more than one place <sup>2</sup>Landessanitätsdirektion für Oberösterreich

<sup>3</sup>National Surveillance Centre = National Reference Laboratory
 <sup>4</sup>Cclin., Lab Merieux Lyon, Service demandeur
 <sup>5</sup>Infection Control Nurse
 <sup>6</sup>Publich health reference centre, regional epidemiological centre

<sup>7</sup>Health Board

	Campylobacter	•	Prepare routinely the plates (n = 616)		Controls the quality of plates (n = 616)					
	laboratories	(n			′es	Ν	lo			
	n	n	%	n	%	n	%			
Austria	41	6	15	21	51	20	49			
Denmark	7	1	14	4	57	3	43			
<b>England &amp; Wales</b>	6	4	67	4	67	2	33			
Finland	21	20	95	21	100	0	0			
France	217	10	5	30	14	184	86			
Germany	138	49	36	88	65	48	35			
Greece	6	6	100	4	67	2	33			
Ireland	35	7	21	18	51	17	49			
Italy	115	24	21	76	67	38	33			
Portugal	13	0	0	4	31	9	69			
Scotland	23	13	57	21	91	2	9			
TOTAL	622	140	23	291	47	325	53			

Table 48. Agar plate preparation and quality control in *Campylobacter* laboratories in 10 European countries, Survey II, 2000

	Campylobacter						
	laboratories	Total r	eplied	Y	es	N	lo
	n	n	%	n	%	n	%
Austria	41	40	98	26	65	14	35
Denmark	7	6	86	6	100	0	0
England & Wales	6	6	100	6	100	0	0
Finland	21	21	100	21	100	0	0
France	217	145	67	53	37	92	63
Germany	138	135	98	19	14	116	86
Greece	6	5	83	0	0	5	100
Ireland	35	19	54	11	58	8	42
Italy	115	109	95	53	49	56	51
Portugal	13	13	100	10	77	3	23
Scotland	23	23	100	23	100	0	0
TOTAL	622	522	84	228	44	294	56

#### Table 49. Participation in external QA schemes for *Campylobacter* culturing in 10 European countries, Survey II, 2000

	<i>Campylobacter</i> laboratories	Total	contiad	V	es		lo	Don'	know	
		<u> </u>	replied %		<del>:</del> 5 %	n	10 %		Don' t know n %	
	n			n						
Austria	41	40	98	22	55	10	25	8	20	
Denmark	7	6	86	4	67	1	17	1	17	
England & Wales	6	6	100	6	100	0	0	0	0	
Finland	21	21	100	19	90	2	10	0	0	
France	217	195	90	37	19	44	23	114	58	
Germany	138	133	96	20	15	47	35	66	50	
Greece	6	6	100	0	0	5	83	1	17	
Ireland	35	35	100	33	94	2	6	0	0	
Italy	115	110	96	48	44	16	15	46	42	
Portugal	13	13	100	9	69	2	15	2	15	
Scotland	23	23	100	23	100	0	0	0	0	
TOTAL	622	588	95	221	38	129	22	238	40	

Table 50. The knowledge about the external quality assurance schemes for *Campylobacter* culturing in 10 European countries, Survey II, 2000

	<i>Campylobacter</i> laboratories	Rer	olied	Accred	litatio	n
	n	n	%	<u></u>	%	Comments
Austria	41	37	90	0	0	1 certification
Denmark	7	6	86	0	0	
England & Wales	6	6	100	3	50	3 CPA <sup>1</sup>
Finland	21	21	100	5	24	5 FINAS <sup>2</sup>
France	217	196	90	0	0	
Germany	138	134	97	11	8	2 CAP <sup>3</sup> , 7 DACH <sup>4</sup> , 2 ZLG <sup>5</sup>
Greece	6	6	100	0	0	
Ireland	35	35	100	0	0	
Italy	115	97	84	0	0	7 certifications
Portugal	13	12	92	0	0	
Scotland	23	23	100	18	78	18 CPA
TOTAL	622	573	92	37	6	

Table 51. Accredited laboratories for	Campvlobacter culturing	a in 10 European countries	(n = 573). Survey II. 2000

<sup>1</sup> Clinical Pathology Accreditation
 <sup>2</sup> Finnish Accreditation Service
 <sup>3</sup> College of American Pathologists, USA
 <sup>4</sup> Deutsche Akkreditierungsstelle Chemie GmbH
 <sup>5</sup> Zentralstelle der Länder für Gesundheitsschutz bei Medizinprodukten

Table 52. Number of primary laboratory findings and notified Campylobacter cases in 1999 in 9 Europea	n countries,
Survey II, 2000.	

	<i>Campylobacter</i> laboratories	All po labora findi	atory	No of notified Campylobacter cases	Report findings		Do not repor findings	
	n	n	sum		n	%	n	%
Statutory surveillance								
Austria	41	34	6199	3188	20	49	21	51
Denmark	7	7	4871	4164	6	86	0	0
Finland	21	21	3664	3303	21	100	0	0
Germany	138	122	24464	28882 <sup>1</sup>	126	91	12	9
Greece	6	6	364	306	5	83	1	17
TOTAL	213	190	39562		172	81	34	16
Sentinel surveillance								
France	217	145	1405	n.r. <sup>2</sup>	24	11	181	83
Ireland	35	18	1193	2085	20	57	13	37
Italy	115	96	2629	n.r.	24	21	89	77
Scotland	23	18	5057	5861	23	100	0	0
England & Wales	6	6	2820	54994	6	100	0	0
TOTAL	396	283	13104		82	21	283	71
ALL	609	473	52666		254	42	317	52

<sup>1</sup> Only 11 Federal States have reported <sup>2</sup> not reported

 Table 53. European countries (n=13) with recommended microbiological procedures for routine laboratory diagnosis of Campylobacter infections, Survey I, 2000

	Recommended procedures	Description / sources of recommended guidelines	Recommended by
Austria	Yes	Selective medium with antibiotics	Workshop by BBSU <sup>1</sup>
Belgium	No		
Denmark	Yes	Direct seed of saline stool suspension on mCCDA with reading after 2 days incubation at $37$ °C in a microaerobic a tmosphere	NRL
France	Yes	Karmali's agar <sup>2</sup>	NRL
Germany	Yes	MiQ 9 / 2000: charcoal-based selective medium with cefoperazon and vancomycin with or without blood	NSS <sup>3</sup>
Greece	No		
Iceland	Yes		National University Hospital
Italy	Yes	Selective medium	NPHI and NSS
Luxembourg	No		
Norway	Yes	Direct culture on mCCDA, incubated in a microaerobic atmosphere at 42°C for 2 days	NPHI and consensus meeting with medical microbiological laboratories <sup>4</sup>
Sweden	Yes	Revision ongoing	NPHI
Switzerland	No		
United Kingdom	Yes	Blood agar (BA), fastidious anaerobe agar (FAA) or CCDA, incubation at 35-37° in microaerobic atmosphere for 40-48 h	NPHI

<sup>1</sup>Bundesstaatlichen Bakteriologisch-Serologischen Untersuchungsanstalten, workshop in 25.-26.2.1999

<sup>2</sup>Megraud F. Methodes Diagnostiques pour les infections a *Campylobacter* d'origine intestinale. Médecine et Maladies Infectieuses 1989;19:12-17 <sup>3</sup>National Scientific Society

<sup>4</sup>Lassen J, Hovig B, Sandven P. Strategimøte nr 10, 1996: Bakteriologiske faecesundersøkelser. Folkehelsa 1997

### Table 54. Recommended (national level) and performed pre-culturing procedures (local level) for Campylobacter investigations in six European countries, Survey I and II, 2000.

		Routine pre-culturing procedures used in primary laboratories									
	Recommendation for pre-culturing procedures on national level	Direct mic	croscopy	Enrich	ment	Othe proced					
	•	n	(%)	n	(%)	n	(%)				
Austria n = 41	No	0	(0)	0	(0)	0	(0)				
Denmark n = 7	Saline stool suspension	0	(0)	0	(0)	1 <sup>1</sup>	(14)				
France n = 217	Enrichment for asymptomatic cases and when the time interval between symptoms and investigation is long	160	(74)	51	(24)	2 <sup>2</sup>	(1)				
Germany n = 138	Enrichment for samples were the amount of bacteria is probably small (long sample transport, healthy carriers)	4	(3)	5	(4)	2 <sup>3</sup>	(1)				
ltaly n = 115	Macroscopic and microscopic stool examination	15	(13)	10	(9)	10 <sup>4</sup>	(9)				
England & Wales n = 6	No	0	(0)	0	(0)	0	(0)				

<sup>1</sup>Suspension in selenite broth and immediate plating on CCDA <sup>2</sup>Homogenisation for quantitative cultivation (1), Sedimentation (1)

<sup>3</sup>Sedimentation (1 laboratory), *Campylobacter*-EIA (1 laboratory)

<sup>4</sup>Sedimentation

Table 55. Recommended procedures for Campylobacter culturing on national level and the<br/>procedures reported by primary laboratories in six European countries, Survey I and II,<br/>2000.

	Recom	mended procedu	ures <sup>1</sup>	Procedures in	primary labo	oratories
	Medium	Inc.temp.ºC/ atmosphere	Inc. time	Media (% of labs using medium)	Inc.temp.ºC range	Inc.time/h range
		-		Campylosel (37%)	37-42ºC	48-168 h
				CampyBap (17%)	42ºC	48 h
				Preston (12%)	37-42ºC	48-72 h
Austria	Selective	42°C/		mCCDA (10%)	42ºC	48 h
(n=41)	medium with	microaerobically	min. 36 h	Karmali/CSM (7%)	42ºC	48 h
	antibiotics	,		Skirrow (5%)	42°C	48 h
				Butzler (2%)	42°C	48 h
		a <b>-700</b> /		CAT (2%)	37ºC	48 h
Denmark (n=7)	mCCDA <sup>4</sup>	37°C/ microaerobically	2 days	mCCDA (100%)	37-42ºC	48 h
				Campylosel (83%)	37-42ºC	24-120 h
France				Karmali/CSM (10%)	37-42ºC	48-120 h
(n=217)	Karmali	37ºC	2 days	Butzler (3%)	37-42ºC	48-72 h
()				CampyBap (1%)	n.r. <sup>7</sup>	n.r.
				Skirrow (1%)	37-42°C	48-72 h
	Charcoal-			mCCDA (17%)	37-42ºC	44-120 h
	based			Butzler (17%)	37-42ºC	44-72 h
	selective medium with			Karmali/CSM (16%)	37-42ºC	48-144 h
Germany		37°C or 42°C/	0. 441	Skirrow (15%)	37-42°C	44-96 h
(n=138)	cefoperazon	5-7% O <sub>2</sub> , 85%	Ca. 44 h	CampyBap (14%)	37-42°C	48-72 h
	and vancomycin,	N <sub>2</sub> , 10% CO <sub>2</sub>		Campylosel (7%)	37-42°C	48 h
	with or			Preston (6%)	37-42°C	48-72 h
	without blood			Blaser-Wang (5%)	37-42°C	24-48 h
				CAT (1%)	37°C	48 h 48-72 h
				Karmali/CSM (23%)	37-42ºC 37-42ºC	24-72 h
				CampyBap (17%) mCCDA (12%)	37-42°C 37-42°C	48-72 h
				Preston (11%)	37-42°C 37-42°C	48-72 h
Italy	Selective	37ºC /	48 h	Skirrow (10%)	37-42°C	48-72 h
(n=115)	medium	microaerobically	40 11	Blaser-Wang (9%)	37-42°C	48-168 h
				Campylosel (9%)	37-42°C	24-96 h
				CAT (4%)	37-42°C	48-72 h
				Butzler (3%)	37-42°C	48 h
	Blood agar	35-37°C/ microaerobicall		mCCDA (33%)	37-42°C	72-96 h
England &	(BA), fastidious	y or anaerobically	40-48 h	Preston (33%)	42ºC	48 h
Wales (n=6)	anaerobe agar (FAA) or	(BA,FAA)	(+ 24 h if required)	Karmali/CSM (17%)	42ºC	48 h
	CCDA	microaerobically (CCDA)		CAT (17%)	37ºC	n.r.

<sup>1</sup>See the table 54.

 Table 56. Recommendations for confirmation of Campylobacter isolates on national level and confirmation practices in primary laboratories in six European countries, Survey I and II, 2000.

	Recommendations for confirmation on national level	labora	o. of atories %)						
		performing confirmation		Microscopy	Oxidase	Catalase	Latex- agglutination	Other reported tests	
Austria (n=41)	Microscopy (morphology) Catalase Oxidase	39	(95%)	97%	87%	54%	22%	Api Campy Resistance pattern	
Denmark (n=7)	Not stated	7	(100%)	100%	86%	86%	0%	No	
France (n=217)	Microscopy (motility) Gram stain Oxidase	187	(86%)	98%	87%	68%	0%	Api Campy Antibiogram Gram-staining Hippurate test	
Germany (n=138)	Not stated	136	(99%)	97%	95%	74%	9%	Accu Probe Aerobic growth Api Campy Resistogram	
ltaly <sup>1</sup> (n=115)	Gram stain Oxidase Catalase Urease Nitrate reduction Hippurate test $H_2S$ production Susceptibility to nalidixic acid and cephalotin Growth at 42°C	111	(97%)	95%	87%	73%	31%	Api Campy Susceptibility to nalidixic acid and cephalotin	
England & Wales <sup>2</sup> (n=6)	Colonial appearance Gram stain Growth in oxygen Oxidase test	6	(100%)	100%	67%	0%	0%	Aerobic growth Temperature tests	

<sup>1</sup>Tests are recommended for identification. Confirmation and species differentiation not separately specified <sup>2</sup>Preliminary identification 

 Table 57. Antimicrobial susceptibility testing methods in NRLs and in primary Campylobacter laboratories in 10 European countries,

 Survey I and II, 2000.

	Method for antimicrobial susceptibility testing in NRL (R=routine)	Primary laboratories that perform susceptibility testing		Reported methods for susceptibility testing in primary laboratories (%)		
				Agar diffusion	E-test	Agar dilution
		n	(%)			
Austria	Agar diffusion (R) E-test	30	(73)	87%	17%	0%
Denmark	Agar diffusion (R) E-test	2	(29)	50%	0%	0%
Finland	No NRL	14	(67)	100%	7%	0%
France	Agar diffusion (R)	103		73%	0%	4%
Germany	Agar diffusion (R)	79	(57)	96%	5%	0%
Greece	E-test (R) Agar dilution	6	(100)	100%	17%	0%
Ireland	No NRL	17	(49)	100%	6%	0%
Italy	Agar diffusion (R)	73	(63)	86%	8%	0%
Portugal	NoNRL	1	(8)	100%	0%	0%
Scotland	See England & Wales	13	(57)	77%	8%	0%
England & Wales	E-test Agar dilution	4	(67)	100%	0%	0%

**Annex II: Questionnaires**