Protocol for a prospective cohort study exploring the gut microbiota of infants with congenital heart disease undergoing cardiopulmonary bypass (the GuMiBear study)

Claire Magner, Dominic Jenkins, Fatma Koc, Mong Hoi Tan, Molly O’Toole, Jordan Boyle, Niamh Maguire, Sophie Duignan, Kiera Murphy, Paul Ross, Catherine Stanton, Colin J McMahon

ABSTRACT

Introduction The gut microbiota develops from birth and matures significantly during the first 24 months of life, playing a major role in infant health and development. The composition of the gut microbiota is influenced by several factors including mode of delivery, gestational age, feed type and treatment with antibiotics. Alterations in the pattern of gut microbiota development and composition can be associated with illness and compromised health outcomes.

Infants diagnosed with ‘congenital heart disease’ (CHD) often require surgery involving cardiopulmonary bypass (CPB) early in life. The impact of this type of surgery on the integrity of the gut microbiome is poorly understood. In addition, these infants are at significant risk of developing the potentially devastating intestinal condition necrotising enterocolitis.

Methods and analysis This study will employ a prospective cohort study methodology to investigate the gut microbiota and urine metabolome of infants with CHD undergoing surgery involving CPB. Stool and urine samples, demographic and clinical data will be collected from eligible infants based at the National Centre for Paediatric Cardiac Surgery in Ireland. Shotgun metagenome sequencing will be performed on stool samples and urine metabolomic analysis will identify metabolic biomarkers. The impact of the underlying diagnosis, surgery involving CPB, and the influence of environmental factors will be explored. Data from healthy age-matched infants from the INFANTMET study will serve as a control for this study.

Ethics and dissemination This study has received full ethical approval from the Clinical Research Ethics Committee of Children’s Health Ireland, GEN/826/20.

INTRODUCTION

What is currently known The establishment of gut microbiota begins at birth and continues over the first years of life. Continued evolution of the gut microbiome after birth is governed by host factors such as both the adaptive and innate immune system, as well as external factors such as diet, medication and toxin exposure, and illness. Understanding the role of the gut microbiome in metabolism, immune function and nutrition is gaining increasing recognition, as it is accepted that an altered colonisation has been associated with a higher risk of disease later in life. In the critical first weeks and months of life, perturbations to the infant gut microbiome have implications for growth development and health.

The microbiome and systemic inflammation It is evident that under certain conditions, disruption of the normal microbiota that colonise the intestinal tract can occur. These conditions include systemic inflammatory processes, which can result in intestinal inflammation, where proinflammatory bacteria can flourish, interacting with the intestinal epithelium to cause cytokine release, activating key inflammatory pathways.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The GuMiBear study will collect microbiome data from children with congenital heart disease at seven time points over a 2-year time frame.
⇒ The study design incorporates comparison with age-matched healthy controls, allowing insights into clinically relevant microbiome alterations throughout and beyond the surgical course.
⇒ The study involves urine metabolomic analysis providing novel insights into the metabolite profile of study participants compared with healthy age-matched infants.
⇒ The primary limitation of GuMiBear is that it is a single-centre study limiting the generalisability of the findings.
increasing morbidity and prolonging critical illness. The pattern of cytokine release in patients undergoing cardio-pulmonary bypass (CPB) is described as comparable to those released in systemic inflammation such as trauma and sepsis. However, the nature of gut microbiota compositional changes in infants undergoing surgery with CPB remains understudied. This research aims to address this knowledge gap to enhance our understanding and inform care practices.

**Congenital heart disease and necrotising enterocolitis**

Congenital heart disease (CHD) affects approximately 1 in every 100 babies born throughout the USA every year. It is the most common congenital defect worldwide. A diagnosis of ‘complex CHD’ can include conditions such as hypoplastic left heart syndrome (HLHS); hypoplastic right heart syndrome, transposition of the great arteries requiring intervention in the first week of life, while CHD such as atrioventricular septal defect, tetralogy of Fallot may require corrective surgery in the first few months of life. CHD requiring surgery involving CPB presents a greater risk to patients. This increased risk is not limited directly to the surgery, compromised ventricular function or low cardiac output state, but includes the risk of developing necrotising enterocolitis (NEC).

There is a well-established connection between CHD and NEC, a potentially devastating intestinal condition of infancy. NEC carries a reported incidence of between 3% and 9% in infants with CHD with all-cause mortality rates as high as 38% in children with ‘cardiogenic’ NEC. While CHD remains one of the most common risk factors for NEC, the underlying pathophysiology of this association is complex. Growing evidence suggests that perturbations in the early-life gut microbiota composition increase the risk for NEC. A significant association between episodes of low cardiac output and shock in the development of NEC is recognised. It is reported that infants with certain types of CHD, mainly HLHS, may possess abnormal systemic vasculature contributing to the increased risk for NEC. Whether it is those, or other causes of impaired perfusion to the gut, the resulting damage to the mucosal barrier can provide an entry point to bacteria provoking an inflammatory cascade and the devastating consequences that can ensue. The vulnerability of infants with CHD is enhanced during the course of surgical intervention involving CPB, and the role of the gut microbiome has received little research focus in this context.

**Surgery involving CPB**

Infants diagnosed with CHD are at risk of alterations to their intestinal homeostasis, a further threat is presented in the context of surgery involving CPB. There is evidence to suggest intestinal ischaemia reperfusion injury occurs after CPB and contributes to epithelial barrier dysfunction (EBD) potentially exposing the bloodstream to bacteria or bacterial products. Although alterations in gut barrier integrity and resident microbiota have been demonstrated, it is not fully understood what changes to the microbiome occur following CPB, and the nature and severity of EBD. While the gut microbiota in infants with CHD following CPB remains understudied, a small single centre case–control study recently identified significant gut microbiota perturbations in patients with CHD. This case–control study highlighted that children with CHD had a disrupted gut microbiome at baseline with an over-representation of proinflammatory bacteria, this was further exacerbated by CPB. Samples were collected preoperatively and in a limited 24 and 48 hours time frame postoperatively. The significance of intraoperative variables including aortic cross clamp time and duration of CPB was not determined.

Our study proposes to address the knowledge gap and advance existing research by examining the gut microbiome of infants with CHD preoperatively, and at defined time points up to 2 years of life/postoperatively. This timeline will account for the recovery phase post cardiac surgery, including time to re-establish full feeds, wean from mechanical ventilation and circulatory support, and allow for surveillance of outcome measures including NEC, repeat surgery and mortality postoperatively. Comparisons will be made with healthy age-matched infants recruited as part of the INFANTMET study. As well as collecting intraoperative variables such as duration of CPB and aortic cross clamp time, a novel aspect of this research will be to profile the metabolites in urine to assess potential metabolic biomarkers and pathway changes. Our research will recruit patients in a National Centre for Paediatric Cardiac Surgery, where 40 open cardiac surgeries are performed on infants annually. We; therefore, anticipate active recruitment will ensure the proposed target sample of 50 participants is achievable. No additional invasive procedures will be required for sample collection, enhancing the acceptability of the research for consenting parents or carers. This project will investigate the subdivisions of the gut microbiota of infants with CHD, and environmental factors such as the influence of mode of delivery, preoperative fasting states and mode of feeding, and use of preoperative antibiotics. Understanding the status of the intestinal microbiome of infants with CHD and the effects of undergoing surgery with CPB is vital in informing best care practices to enhance patient outcomes.

**Objectives and outcomes**

The primary study objectives and outcomes are:

- To characterise the gut microbiota composition of infants with specified CHD undergoing surgery with CPB at specific time points perioperatively.
- To compare any differences in gut microbiota composition of infants who take part in this study at defined time points preoperatively and postoperatively and compare with the microbiota of healthy babies from the INFANTMET study at matching time points.
- To characterise the urine metabolite profile of infants with specified CHD undergoing surgery with CPB and
compare with healthy infants from the INFANTMET study.\textsuperscript{19}

Secondary objectives and outcomes:
To explore the influence of maternal and environmental factors on gut microbiome composition.

**METHODS**

**Study design**
This study is a prospective cohort study of infants with CHD undergoing CPB at the National Centre for Paediatric Cardiac Surgery at Children’s Health Ireland (CHI) at Crumlin, Dublin, Ireland. This single-site study will investigate the differences in the gut microbiome, metabolomics readouts and stress levels between infants with CHD undergoing CPB and healthy age-matched controls.

**Participant selection**
This study will involve the collection of demographic and clinical maternal and infant data from infants diagnosed with CHD scheduled for surgery involving CPB. CHD is typically diagnosed antenatally with fetal echocardiography performed routinely at 20–24 weeks gestation. Any child presenting with a murmur or features of cardiac conditions, or symptoms of CHD will be diagnosed using clinical examination, including palpation, auscultation, ECG and echocardiography. Cardiac diagnoses are classified according to cardiovascular physiology, that is, left to right shunt, cyanosis with biventricular circulation, univentricular circulation and outflow tract obstruction, as presented in [table 1].

To be eligible for the study, the participants must meet the terms of the inclusion and exclusion criteria as presented in [table 2].

**Public and patient involvement statement**
The mother of a child who had cardiac surgery as a baby was involved in reviewing the research questions, outcome measures and study literature at the study design phase. The public and patient involvement (PPI) representative did not participate in the recruitment or the conduct of the study due to competing demands and availability. The PPI representative has offered to support dissemination of the study results through their involvement in charitable foundations and child and parent support fora.

**Recruitment**
Participants meeting all inclusion criteria will be selected after admission to the hospital, outpatient clinic or cardiac day unit. Study-related information will be given in written form as well as explained by a member of the project team. No study-related activities will begin before the potentially eligible participants’ parents/carers have signed the informed consent form (ICF). Participants parents/carers will be asked to refer to the privacy notice on the hospital website or they can receive a hardcopy

### Table 1 Classification of CHD subtypes

<table>
<thead>
<tr>
<th>Group</th>
<th>CHD group</th>
<th>CHD subtypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Left to right shunt</td>
<td>AVSD, VSD, large ASD</td>
</tr>
<tr>
<td>2</td>
<td>Cyanotic CHD with biventricular circulation</td>
<td>D-TGA/IVS, D-TGA/VSD</td>
</tr>
<tr>
<td>3</td>
<td>Cyanotic CHD with univentricular circulation</td>
<td>HLHS, HRHS (Tricuspid Atresia) Pulmonary Atresia</td>
</tr>
<tr>
<td>4</td>
<td>RVOT obstruction</td>
<td>Tetralogy of Fallot, dysplastic PS</td>
</tr>
<tr>
<td>5</td>
<td>LVOT obstruction</td>
<td>Shones syndrome, coarctation of aorta, interrupted aortic arch</td>
</tr>
<tr>
<td>6</td>
<td>Others</td>
<td>Ebstein anomaly, truncus arteriosus</td>
</tr>
</tbody>
</table>

ASD, atrial septal defect; AVSD, atrioventricular septal defect; CHD, congenital heart disease; CPB, cardiopulmonary bypass; DTGA, D-transposition of the great arteries; DTGA-IVS, D-transposition of the great arteries with intact ventricular septum; HLHS, hypoplastic left heart syndrome; HRHS, hypoplastic right heart syndrome; LVOT, left ventricular outflow tract; PS, pulmonary stenosis; RVOT, right ventricular outflow tract; VSD, ventricular septal defect.

### Table 2 Inclusion and exclusion criteria

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants born full term (≥37 weeks gestation)</td>
<td>Stillbirth or live birth where the baby is born alive but dies shortly after</td>
</tr>
<tr>
<td>Infants diagnosed with *CHD and scheduled for surgery involving CPB</td>
<td>Infants who are born healthy with no underlying illness, syndrome, or chronic disease</td>
</tr>
<tr>
<td>Infants born in Ireland to allow sample follow-up</td>
<td>Participation in another study</td>
</tr>
<tr>
<td>Ability of the participant’s parent/carer (in the investigator’s opinion) to comprehend the full nature and purpose of the study</td>
<td>Infants not undergoing surgery involving cardiopulmonary bypass</td>
</tr>
<tr>
<td>Consent to participate in the study and willingness to comply with the protocol and study restrictions by the participant’s parent/carer</td>
<td>Infants where parents/carer do not give consent to participate in the study</td>
</tr>
</tbody>
</table>

*CHD diagnoses and subtypes are presented in table 1. CHD, congenital heart disease; CPB, cardiopulmonary bypass.
if they wish. Signed ICFs will be stored safely in a locked cabinet in the research office.

Compensation
No compensation will be provided to the participants. There are no cost implications for the health service executive or to the participants. The management of patients and investigative tests will comply with current standards of care.

Study timeline
After completing recruitment procedures, that is, determining whether the patient meets the study inclusion criteria, discussing the study with the parents/caregivers and obtaining informed consent, clinical and demographic data will start to be collected. The study will be undertaken for a period of 2 years after the infant is initially recruited.

Demographic data
The infants’ diagnosis including whether antenatal or postnatal diagnosis, comorbidities, date of birth, gestational age, sex, mode of delivery, Appearance, Pulse, Activity, Grimace, Respiration scores, birth weight, head circumference, mode and type of feeding pre and post operatively, antibiotics administered postdelivery, complications and antenatal events will be recorded (case record form located in online supplemental appendix 1).

Maternal data
Maternal history including age, antibiotics received, smoking status use of probiotics and significant antenatal events will be recorded.

Surgical course
The type of surgery performed, duration of CPB and cross-clamp time will be recorded. Antibiotic use and any intraoperative events will be recorded as well as clinical information including arterial blood gas data.

Postoperative data
Paediatric Index of Mortality score, duration of mechanical ventilation, haematology variables including haemoglobin and haematocrit, renal data, for example, blood urea nitrogen and creatinine, fluid balance and cardiovascular support including vasoactive inotrope score, as well as duration of stay in paediatric intensive care unit will be recorded.

Feeding information data
Feeding information including the type of feed and duration of the feed prior to and after surgery will be recorded. The date the patient is established on full feeds will be recorded. Full feeding is defined as when the patient no longer requires parenteral nutrition or intravenous fluids.

Discharge information data
Discharge information data: This will include the patient’s status on discharge from paediatric intensive care unit (PICU), as well as length of PICU and hospital stay.

Complications
The occurrence of complications will be recorded, for example, the development of NEC. The timeline for recording NEC onset will be based on the initiation of triple antibiotic therapy, based on a full surgical review including clinical presentation, radiological and laboratory data. In addition, the occurrence of death, rehospitalisation for heart failure or cardiac problems will be included.

Subject withdrawal/exclusion
Under the Declaration of Helsinki, the research nurse will explain to the consenting adult that they have the right to withdraw from the study at any time and that this will in no way prejudice their future treatment. The reason for withdrawal will be recorded in the source documents and on the appropriate CRF. Consenting adults will be made aware that stored samples from individuals withdrawing from the study may have undergone processing and may be analysed in the study.

Regulatory procedures
The study is conducted following the version Fortaleza, Brazil, October 2013 of the Declaration of Helsinki 1964. The Protocol and the ICF have been approved by the Clinical Research Ethics Committee of Children’s Health Ireland GEN/826/20. As biological samples will be procured in one institution and sent to another, a data-sharing agreement is in place between The Cardiology Department, CHI at Crumlin Hospital, and APC Microbiome Ireland, in Cork. This research is fully compliant with the guidelines as set out in The General Data Protection Regulation, the Irish Data Protection Acts 1988 to 2018 including Protection Act 2018 (Section 36(2)) (Health Research) Regulations 2018.

Data statement
Once collected, the anonymised demographic, clinical and laboratory analysis data as well as statistical codes will be uploaded to the open access Research Repository University College Dublin.

Sample collection and analysis
Faecal samples
Stool samples will be collected at the following time points: within 24 hours after birth (timepoint (TP) 1), within 24 hours presurgery (TP 2), 1 week postsurgery (TP 3) 4 weeks postsurgery (TP 4), 24 weeks postsurgery (TP 5) at 52 weeks (TP 6) and 2 years of age (TP 7). Information about antibiotic therapy administered before or during the stool collection will be recorded.

As the study site is the National Centre for Paediatric Cardiology, all infants diagnosed with CHD antenatally are transferred to the study site from the Maternity Unit for management of CHD. The first study stool specimen is typically obtained after the infant is transferred to the study site and informed consent obtained, which is typically within 24 hours of delivery.
The sample will be collected by the bedside nurse or the parent/carers and transferred to the laboratory on receipt of the sample during the weekdays or weekends. At night, the sample will be kept in the dedicated fridge and transferred to the laboratory within 4–5 hours for appropriate storage at −80°C until further analysis. A Standard Operating Procedure for sample collection when participant is no longer an in-patient is provided in online supplemental appendix 2.

**Urine samples**

Testing the urinary metabolomics of study participants will allow the potential identification of altered metabolomic profiles, and explore the role of microbiota in such alterations. Mirroring the INFANTMET study, urine samples will be collected at 4–8 weeks postsurgery for metabolomic analysis using Sterisets Uricol Urine Collection Pack (MedGuard, Ireland). The urine sample will be collected from the urinary catheter if the participant is catheterised. Alternatively, a pad will be placed in the diaper and used to collect an unsoiled urine sample from the infant. The pad will then be placed in a biohazard bag and frozen immediately at −80°C prior to processing. After all the sample collections are complete, they will be shipped to Teagasc Food Research, Moorepark, Ireland, using DHL overnight service for microbiome and metabolomics analyses. Styrofoam Safe-T-Pak STP-309 shipper box or equivalent will be used. DNA extraction will be performed on stool samples using the modification of the Repeated Bead Beating Plus Column method. LC-MS (liquid chromatography–mass spectrometry) will be used for metabolomics analysis of urine.^{19}

**Sample collection for discharged participant**

Parents/carers will receive a sample collection discharge pack and a parent diary/instruction served as a reminder to collect due samples at different time points prior to discharge home. They will receive a follow-up text message or phone call to remind them on the due sample. The sample collection discharge pack consists of urine/stool collection containers with study code, sterile pad, syringe, zip-lock bag, gloves, biohazard bag and cooler bag. Parents/carers are asked to keep the collected sample at the dedicated section of their home freezer. They will transport the collected sample in the cooler bag provided when attending outpatient department for appointments. They will ask a member of the project team to transfer the collected sample to the laboratory on arrival at the hospital. The study researcher is available at the dedicated contact number for any queries.

**Adverse events and participant well-being**

There are no expected safety concerns related to the study. All study participants will be under the care of the cardiology team at the hospital with access to psychological support, as well as nursing and medical professionals, social workers and chaplaincy.

**Data collection and management**

The study diaries, study dataset and paper/digital CRF systems will be used for recording data from each study participant. All the data collected in this study are pseudonymised, as each of the participants will be assigned a specific study code and on receipt, data will be referred to the study code. All study staff responsible for entering data into the CRF system received training in advance of the study commencement. This training included familiarity with the study diaries, study dataset and paper/digital CRF system and have completed good clinical practice in research training. They have individual access to the password protected study shared drive within the hospital. The study team will monitor the data/sample collection process. Any inconsistencies identified during the study will be presented as queries at the regular project team meeting.

**COMPARISON DATA**

Data collected as part of the INFANTMET Study will serve as a healthy control comparison for this study. INFANTMET compared the gut microbiota development of breastfed infants born via C-section or vaginally at full term or preterm at Cork University Maternity Hospital. Ethical approval for sample collection by Cork University Hospital Research Ethics Committee (reference number ECM(w) 07/02/2012). One hundred and ninety-two infants were recruited to the INFANTMET study and stratified according to delivery mode and gestational age at birth. Faecal samples were collected from the infants at 1, 4, 8 and 24 weeks of age and years 1, 2 and 4 of life and stored under controlled conditions. Urine samples were collected at 4 weeks of age for metabolomic analysis and stored in a freezer at −80°C prior to processing. Samples were analysed in accordance with the analysis proposed for the GuMIBear study. Although INFANTMET study participants did not have CHD, they nonetheless serve as a valuable comparison group. The stool and urine samples collected as part of the GuMIBear study will be as closely time matched as possible to the INFANTMET study samples to capture the major developmental period of the early life gut microbiota.

**Bioinformatics and statistical analysis**

**Sample size justification**

Published research in this area is lacking. However, one case–control study by Salomon et al^{18} included 17 cases and 12 control participants and was sufficiently powered to determine a statistically significant difference in beta diversity in cases versus controls (F=5.6, p<0.001). Our study proposes to include 50 patients with CHD undergoing surgery with CPB and age matched controls, almost three times the Solomon et al study. We; therefore, anticipate that the proposed sample size is justified.
DISCUSSION

Despite an increasing awareness of the early-life critical window of microbiome development on the health and wellness of infants, there remains much to learn about the interactions of the microbiome with the infant host with CHD undergoing surgery involving CPB. This study is designed to address this knowledge gap, and incorporates a sound methodology with particular strengths enhancing the value of its findings. The specimen collection strategy occurring at multiple time points over a 2-year period in the proposed study will deepen our understanding of the temporal dynamics of the colonising microbiota, and their interactions with host physiology. The study design will account for maternal and perioperative variables to determine changes to the microbiome. Access to existing microbiome data from healthy age-matched infants provides a valuable opportunity to present high-quality comparative information. A limitation of this study may include the failure to recruit infants with CHD not identified antenatally, despite active fetal screening services. While multicentre trials capturing sufficient case numbers of NEC cases will offer robust conclusions, this study will offer valuable evidence to support the influence of CHD and CPB on the microbiome and intestinal EBD. Future research can build on existing studies, and explore treatment strategies including recommendations for efficacious probiotic strain administration, including the supplements to promote a diverse gut microbiota to improve outcomes for this vulnerable population.

Ethics and dissemination

This research study is ethically approved by the Clinical Research Ethics Committee of Children’s Health Ireland (REC REF No: GEN/826/20). Study results will be available to patients with CHD and their families, carers, support networks, paediatric cardiology and microbiome societies and other researchers. Study findings will provide a deeper understanding of the gut microbiota of infants with CHD and inform perioperative management options including strategies to prioritise the integrity of the gut microbiota.

Status of Study

The trial is ongoing and as of 5 February 2023, 84% of the participants have been recruited. Laboratory analysis has been carried out on 25% of study samples.

Author affiliations

1 School of Nursing, Midwifery and Health Systems, University College Dublin, Dublin, Ireland
2 Laboratory, Children’s Health Ireland at Crumlin, Crumlin, Ireland
3 School of Microbiology, University College Cork, Cork, Ireland
4 Food Biosciences, Teagasc Food Research Centre, Cork, Ireland
5 Department Paediatric Cardiology, Children’s Health Ireland at Crumlin, Dublin, Ireland
6 University College Cork APC Microbiome Institute, Cork, Ireland
7 Food Biosciences, Teagasc Food Research Centre, Moorepark, Ireland
8 University College Cork College of Science Engineering and Food Science, Cork, Ireland
9 Teagasc Food Research Centre, Moorepark, Ireland
10 University College Dublin School of Medicine, Dublin, Ireland

Twitter Claire Magner @clairemagner1 and Fatma Koc @fatmakoc21

Acknowledgements All study participants, Paediatric Intensive Care Unit/Children’s Heart Centre nurses for assisting in sample collection. All laboratory staff including Ms Irene Regan who have facilitated tracking and secure storage of study
samples. Dr Adam James, Dr Ross Foley and the Consultant Paediatric Consultants, CHI at Crumlin for their support with this study.

Contributors CM, CJM, CS and KM devised the project, and the main conceptual ideas. CJM, CM, CS, PR, FK, DJ and MHT were involved in the study design and writing of the manuscript. MOT, JB, NM, SD and CJM are involved in consenting participants, collecting samples and acquiring data. DJ, CS, FK and PR are responsible for analysing study samples. All authors read manuscript revisions, approved the final manuscript and accept accountability for the accuracy and integrity of the work.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

Supplemental material This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is permitted others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is not necessary. See: http://creativecommons.org/licenses/by-nc/4.0/.

ORCID iD Claire Magner http://orcid.org/0000-0001-5807-588X

REFERENCES


20 Yu Z, Morrison M. Improved extraction of PCR-quality community DNA from digesta and fecal samples. *Biotechniques* 2004;36:808–12.