Leishmaniasis: new approaches to disease control

Clive R Davies, Paul Kaye, Simon L Croft, Shyam Sundar

Leishmaniasis is one of the major infectious diseases affecting the poorest regions of the world, but new developments in diagnosis, treatment, and control offer some fresh hope

The leishmaniases afflict the world's poorest populations. Among the two million new cases each year in the 88 countries where the disease is endemic (fig 1), it is estimated that 80% earn less than $2 a day. Human infections with *Leishmania* protozoan parasites, transmitted via the bite of a sandfly, cause visceral, cutaneous, or mucocutaneous leishmaniasis. The global burden of leishmaniasis has remained stable for some years, causing 2.4 million disability adjusted life years (DALYs) lost and 59 000 deaths in 2001.[1] Neglected by researchers and funding agencies, leishmaniasis control strategies have varied little for decades, but in recent years there have been exciting advances in diagnosis, treatment, and prevention. These include an immunochromatographic dipstick for diagnosing visceral leishmaniasis; the licensing of miltefosine, the first oral drug for visceral leishmaniasis; and evidence that the incidence of zoonotic visceral leishmaniasis in children can be reduced by providing dogs with deltamethrin collars. There is also hope that the first leishmaniasis vaccine will become available within a decade. Here we review these developments and identify priorities for research.

Summary points

- Simple and rapid diagnostic tools for leishmaniasis will soon be widely available
- A new range of affordable and effective treatments, including oral drugs, are coming on line
- The development of drug resistance is a major concern and needs strategic plans
- New methods of applying insecticides for preventing leishmaniasis are likely to take the place of house spraying and (for zoonotic visceral leishmaniasis) dog culling
- Strategies need to be developed to optimise progression of vaccine candidates to phase I trials
- Current research effort and resources should prioritise visceral leishmaniasis in the Sudan and Indian subcontinent, where the burden of leishmaniasis is greatest

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Methods

This review is based on our personal knowledge of the subject combined with a literature search on the Entrez-PubMed site.

Diagnosis

Visceral leishmaniasis

The gold standard for diagnosing visceral leishmaniasis is parasite identification in tissue smears, with splenic aspirate being more sensitive than bone marrow or lymph node aspirates. However, difficulties in obtaining and examining tissues mean that serological methods are increasingly being used.

Box 1: Current research priorities for diagnosing leishmaniasis

- Develop affordable tools for use in the field (including antigen detection tests) for rapid diagnosis customised for specific geographical regions
- Develop semiquantitative rapid methods for prognosis and early detection of relapse
- Develop probes for detection of infections with drug resistant strains

The direct agglutination test, in which stained parasites are agglutinated by serum antibodies, is popular in Iran and Africa,[w1] but variation between batches and the high cost of commercially available antigen are limiting factors. In the Indian subcontinent,[2] but less so in Europe and Africa,[3] [w2] a rapid strip test is used to detect antibody to rK39 (a conserved antigen of *L infantum*) and is both sensitive (67-100%) and specific (93-100%) (fig 2). Weak responses in some patients, persistence of antibodies after cure, and presence of antibodies in some healthy individuals are inherent limitations with antibody based diagnostics.

Detection of leishmanial antigen in urine through a latex agglutination test (Katex) seems to be promising for both diagnosis and prognosis.[4] Techniques based on polymerase chain reaction are potentially highly sensitive and specific,[5] but they need to be made more suitable for field use in terms of cost and user skills

References w1-w30 are listed on bmj.com

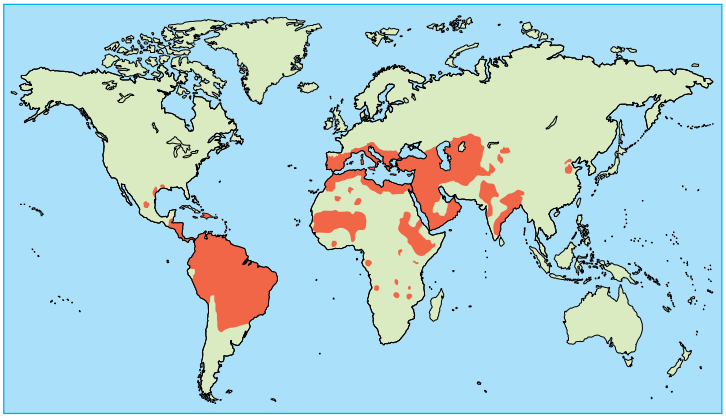

Fig 1 Global distribution of leishmaniasis

required. In patients co-infected with HIV and visceral leishmaniasis, blood smears and culture might yield good results.[w3]

Cutaneous and mucocutaneous leishmaniasis

Touch smears or culture of exudates or scrapings yield good results in the diagnosis of cutaneous leishmaniasis. From a nodule, slit skin smears are often rewarding. Tissue biopsy can be used for impression smears, culture, or animal inoculation, especially for mucocutaneous leishmaniasis. Although multiple *Leishmania* species sometimes coexist, species identification is unlikely to be cost effective in the field unless major treatment decisions for cutaneous leishmaniasis become species specific.

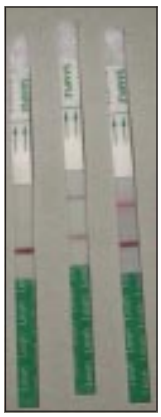

Fig 2 Diagnostic results from three serum samples using the rapid rK39 immunochromatographic dipstick test for diagnosing visceral leishmaniasis. The single band represents a negative control, while the two results with a double band reflect positive diagnoses for patients with visceral leishmaniasis. This user friendly test is now widely used in Nepal and India

Treatment

For six decades, long parenteral courses of pentavalent antimonial (Sb^v) drugs have been used for both visceral and cutaneous leishmaniasis. The second line drugs amphotericin and the less frequently used pentamidine isetionate are toxic. The difficulties of treatment are exacerbated by the spread of resistance to antimony in India[6] and the intractability of the disease to all drugs in patients co-infected with HIV.[w3] In most endemic countries the use of some excellent treatments, notably liposomal amphotericin (AmBisome) for visceral leishmaniasis, is limited by patients' inability to pay.[7]

Visceral leishmaniasis

Concerns about treatment failure for visceral leishmaniasis are exacerbated by geographical variations in antimonial treatment regimens, severity of disease, and sensitivity of *Leishmania* species. In north Bihar (India) there is clear evidence of acquired resistance of *L donovani* to antimonials,[w4] with up to 60% failure rate with treatment.[6]

Amphotericin B has been a standby treatment during this developing crisis[w5]; the drug can be used in short courses and gives >90% cure rate.[7] In addition, the aminoglycoside paromomycin, effective in phase II trials,[8] is likely to be approved after a pivotal phase III study is completed. However, it is the alkylphosphocholine miltefosine (fig 3), first developed as an anticancer drug and which can be taken orally, that offers the most hope. In a series of trials this drug achieved a 94% cure rate at doses of about 2.5 mg/kg (100 mg/day for four weeks) even among patients with antimony resistant disease.[9] [w6] Miltefosine was registered for treating visceral leishmaniasis in India in March 2002. Subsequent trials in children have returned similar results. Because of its teratogenic potential, this drug should be used with caution in women of childbearing age. Another potential oral drug sitamaquine, an aminoquinoline, lacked a linear correlation between dose and cure rates and had an unsatisfactory safety and efficacy profile.[w7]

HIV co-infections with *L infantum* and occasionally cutaneous leishmaniasis have proved difficult to treat, with over 60% failure rate after treatment with most antileishmanial drugs used either alone or in combination.[w8] HAART (highly active antiretroviral therapy) has some effect on the relapse rate.[10]

Cutaneous leishmaniasis

Over 90% of cases of cutaneous leishmaniasis (irrespective of the parasite species responsible) heal spontaneously within 3-18 months, and the rationale for drug use and determination of drug efficacy are different from those for visceral leishmaniasis. A three week course of antimonial drug is the most common treatment, especially in patients with disfiguring or relapsing cutaneous or mucocutaneous leishmaniasis.[w9]

Trials with paromomycin ointments indicate considerable potential for treating cutaneous leishmaniasis (box 2). Imiquimod, an immunomodulator for genital warts, produced 90% cure rate when the ointment was used in conjunction with antimonials in 12 patients with cutaneous leishmaniasis in Peru who had not responded to antimony alone.[11] The potential of this approach is yet to be fully exploited.

Oral drugs have also shown promise. In an open label study in Colombia four weeks' treatment with

Box 2: Topical paromomycin formulations for cutaneous leishmaniasis

- In placebo controlled trials 15% paromomycin+12% methylbenzethonium chloride ointment gave cure rates of 74% in Europe, Asia, and Africa[w10] and 85% in North and South America[w11]
- 15% paromomycin+0.5% gentamicin+10 surfactant vehicle gave cure in 35 days compared with 56 days for placebo[w12]

Fig 3 Children taking miltefosine, the first oral drug for visceral leishmaniasis, which was registered in India in March 2002. Trials indicate that this drug is highly effective, even against antimony resistant cases

Box 3: Current research priorities for treating leishmaniasis

- Develop easy drug sensitivity assays
- Test oral miltefosine in regions other than Indian subcontinent for visceral and cutaneous leishmaniasis and variants
- Complete registration requirement of paromomycin in India, initiate trials in South America and other regions
- Investigate oral sitamaquine in Africa and Indian subcontinent
- Develop cheaper indigenous formulations of lipid amphotericin
- Test multiple drug treatment to protect current and future drugs.

Box 4: Current research priorities for vector and reservoir control

- Develop rapid and accurate diagnostic test for dogs infected with zoonotic visceral leishmaniasis
- Measure impact of community-wide use of insecticide treated dog collars on the incidence of zoonotic visceral leishmaniasis in children in different settings
- Measure impact of using insecticide treated bed nets on leishmaniasis in different settings, including the relative impacts of household protection versus the mass effect
- Test effectiveness of new applications of insecticide—such as cattle sponging against visceral leishmaniasis in Indian subcontinent, insect repellent lamps in regions with high rates of indoor biting at dusk by sandfly vectors, insecticide treated bed sheets and clothing or plastic sheeting for refugee camps
- Develop high resolution maps of leishmaniasis risk and amenability to particular control activities in order to help rationalise the targeting of interventions

miltefosine, 133 mg and 150 mg daily cured 100% and 89% of patients respectively.[12] For 20 years there has been interest in antifungal azoles for treating leishmaniasis, based on both microbial organisms having ergosterol as their predominant sterol. Most trials have been limited, and results are equivocal. Indications from controlled clinical trials are that ketoconazole has some potential against *L mexicana* infection,[w13] and recently fluconazole 200 mg/day for six weeks led to healing of cutaneous leishmaniasis (*L major*) in 79% of patients compared with 34% with placebo.[13]

Vector and reservoir control

Insecticide spraying of houses

Spraying houses with insecticide is the most widely used intervention for controlling sandflies that are endophilic (rest mostly indoors after feeding). House spraying with the pyrethroid lambdacyhalothrin reduced the odds of cutaneous leishmaniasis in Kabul by 60%[14] and reduced the risk of cutaneous leishmaniasis in the Peruvian Andes by 54%.[15] Both trials measured protection at the household level, and it remains unclear under what circumstances "blanket spraying" of all houses in a village would have an additional mass effect on sandfly populations.[w14] Such evaluations are crucial for the cost effective targeting of house spraying. For example, in Sao Paulo State (Brazil) spraying against sandflies is activated by the reporting in a municipality of more than two cases of cutaneous leishmaniasis in a single year.[w15] Sustainability is vital, as cessation of spraying campaigns invariably leads to the re-emergence of leishmaniasis to pre-control levels.[w16]

Bed nets

Where sandflies are endophagic (mainly feed indoors) and most active when people are asleep, bed nets provide considerable protection. For example, a case-control study in Nepal showed that people using untreated nets were 70% less likely to develop visceral leishmaniasis than people without nets.[16] Protection provided by wide mesh nets is enhanced by treating them with pyrethroids—reducing sandfly biting rates by 64%-100%.[w17] [w18] There is also evidence from Colombia that sandfly bites are not diverted to people sleeping outside insecticide treated nets: "unprotected" people in the same room as someone sleeping under a deltamethrin treated net received 42% less sandfly bites than people in houses without nets.[w17] After encouraging, but inconclusive, results from small scale epidemiological trials in Iran,[w19] Syria,[17] and Sudan (D El Naiem personal communication), the household trial in Kabul showed that permethrin treated nets were no less effective than house spraying, reducing cutaneous leishmaniasis risk by 65%.

This result cannot be extrapolated elsewhere, as effectiveness of insecticide treated bed nets is determined by local sandfly behaviour. It is unclear, for example, whether the provision of about 300 000 such nets by Médecins Sans Frontières in eastern Sudan during 1998-2001 substantially reduced the incidence of visceral leishmaniasis (R Davidson personal communication). To our knowledge, insecticide treated nets have not been introduced outside the Sudan by any leishmaniasis control programme (as opposed to a trial) except in Afghanistan and Pakistan, where they have been provided by HealthNet to patients with active cutaneous lesions (*L tropica*). Their aim is not to protect the patients but to block transmission, as *L tropica* is transmitted anthroponotically. As with house spraying, it is unclear whether the widespread use of insecticide treated nets will have any mass effect, as

Fig 4 This deltamethrin treated collar (Scalibor, InterVet, Boxmeer, Netherlands) provides dogs (the reservoir host of *L infantum*) with long term protection against sandfly bites and canine visceral leishmaniasis. Field trials in Iran show that providing all dogs in a community with collars also protects children against *L infantum* infection

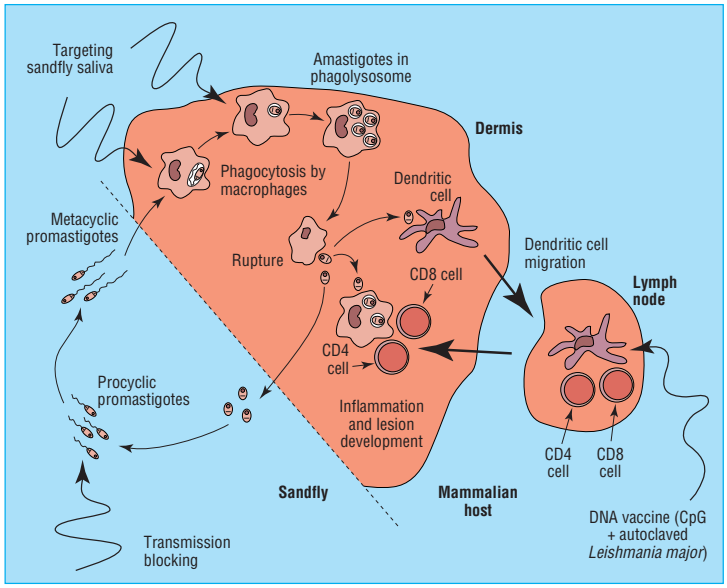

Fig 5 The life cycle of *Leishmania* parasites and targets for vaccination. *Leishmania* cycle between sandfly vectors, where they exist as multiplicative "procyclic" promastigotes and infective "metacyclic" promastigotes, and their mammalian host, where they exist as intracellular amastigotes living predominantly in the phagolysosome of macrophages. After initial infection, amastigotes may replicate for some time before triggering an inflammatory and adaptive immune response. The latter requires migration of dermal dendritic cells to draining lymph nodes and their presentation of antigens derived from *Leishmania* to both CD4 and CD8 T cells. These then accumulate in the developing inflammatory lesion and promote parasite destruction by producing cytokines able to activate macrophage defences. Vaccination may promote these responses if vaccine antigens are delivered in an appropriate way to trigger both T cell subsets. Alternatively, immune responses against sandfly saliva may cause rapid local inflammation not conducive to parasite survival or block the function of salivary immunomodulators. Finally, host immune responses may target essential steps in parasite development within the sandfly (such as attachment to the fly midgut)

there was no detectable impact on sandfly abundance in either the Iranian or Syrian trial.[17] [w19] However, a recent cluster randomised trial in Venezuela showed that village-wide treatment of loosely hanging curtains with lambdacyhalothrin significantly reduced indoor sandfly abundance and eliminated cutaneous leishmaniasis risk.[w20]

Control of zoonotic infection

In Brazil about 200 000 houses are sprayed and 20 000 dogs are culled each year to prevent zoonotic visceral leishmaniasis. After annual surveys in endemic regions, dogs are culled if their blood samples are diagnosed positive by immunofluorescence. Although experimental trials indicate that dog control may reduce *Leishmania* incidence in both dogs and children,[18] concerns over the delays between sampling, diagnosis, and culling, and a failure to reduce the number of notified cases, have led to scepticism of the effectiveness of the Brazilian control programme.[w21] More effective diagnostic tools may allow culling without delay,[w22] but a recent trial of the rK39 dipstick test (see above) showed poor specificity (<75%) for diagnosing infected dogs.[19]

In the absence of a reliable and rapid tool for detecting infected dogs, alternative control strategies for zoonotic visceral leishmaniasis are being sought. Dipping dogs in insecticide[w23] or applying topical insecticide lotions[20] can substantially reduce sandfly bites on dogs and so protect them from infection, but this strategy requires regular retreatment as the insecticidal effect is short lived. However, a novel method for topical application of insecticide on dogs enables the insecticidal effect to persist for up to eight months.[w24] Experimental trials have consistently shown that deltamethrin treated collars (fig 4) reduce by up to 90% the proportion of sandflies that take a blood meal and survive.[20] [w24] Widespread use of these collars with domestic dogs in Italy reduced their risk of being infected by *L infantum*,[w25] and a matched cluster randomised trial in Iran showed that, not only was the odds of infection in dogs reduced by 54%, but children living in the treated villages had significantly less risk of infection as well (odds ratio 0.57).[21]

Box 5: New targets for vaccination—sandfly saliva

- Sandfly saliva contains a variety of immunogenic proteins, including some that enhance parasite survival by inhibiting macrophage function[w28]
- Vaccine induced immunity to saliva stimulates a vigorous delayed type hypersensitivity response after a bite, generating conditions unfavourable for parasite survival[w29]
- The effectiveness of this approach in humans remains to be tested
- Epidemiological studies should determine whether natural immunity to saliva dictates patterns of infection

Box 6: Current research priorities for vaccine control

- Develop a coordinated approach to evaluating vaccine candidate antigens, minimising duplication of effort and maximising use of limited financial resources
- Immunogenicity testing of candidate vaccine antigens in humans, adopting a broad-based analysis of both CD4 and CD8 T cell function
- Further analyse the immune response during "leishmanisation," which offers the first real prospect of understanding the ontogeny of the human immune response
- Develop new challenge models for visceral leishmaniasis, mimicking sandfly infection
- Evaluate vaccines for canine visceral leishmaniasis, for veterinary use and as aids to controlling human disease

Progress in vaccine development

Of all the parasitic diseases, leishmaniasis is considered the most likely to succumb to vaccination. The parasite has a particularly simple life cycle (fig 5), resolution of primary cutaneous leishmaniasis usually results in resistance to re-infection, and studies in experimental models have suggested simple CD4 Th1-type, cell mediated resistance (involving activation of macrophage killing mechanisms by T lymphocyte-derived interferon γ). In experimental models of cutaneous leishmaniasis, in which CD4 Th1 responses are driven towards a polarised Th1 response, protection can indeed be achieved by vaccination, although this rarely results in complete protection from development of lesions. Such vaccines, however, stimulate only poor memory, and protection wanes after a few weeks.[22] In

Educational resources

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- Communicable Disease Surveillance and Response (CSR). Leishmaniasis. www.who.int/emc/diseases/leish (accessed 13 Dec 2002)
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primate studies and clinical trials they show immunogenicity but rarely give appreciable protection.[23 24 w26]

In the past three to four years our view of the requirements for vaccine induced immunity has changed. A major paradigm shift reflects the role of CD8 T cells. New models of cutaneous leishmaniasis indicate that CD8 cells are vital for primary resistance.[24] It has also been recognised that CD8 cells are required for the maintenance of long term vaccine induced immunity. Although the capacity to induce CD8 cell responses is a feature of DNA vaccines, this has also been shown for some protein based vaccines.[25]

Although clinical trials continue with crude autoclaved *L major*, some defined protein antigens have been identified, sometimes rationally, sometimes by serendipity.[22] The *Leishmania* Genome Project (www.sanger.ac.uk/Projects/L_major/) provides an impetus for immunisation with an expression library of the parasite to screen all expressed proteins.[26] Recent advances in combining vaccine delivery systems in so called prime-boost schedules are also being tested in models of cutaneous leishmaniasis.[w27] The bottleneck imposed by producing materials to the standards of good laboratory practice (GLP) and good manufacturing practice (GMP) and the costs of trials are major hurdles in taking any of these discoveries further. A $15m (£9.4m; €15.1m) award from the Bill and Melinda Gates Foundation to support vaccine development at the Infectious Disease Research Institute in Seattle is a welcome boost. A recombinant vaccine (Trifusion, a fusion peptide of the leishmanial antigens LMST11, TSA, and LeIF) is being prepared for clinical trials, and the recent observation that Trifusion, given in combination with immunostimulatory CpG oligodeoxynucleotides, stimulated CD4 and CD8 cells and long term immunity is encouraging.[25]

Rapid progress in vaccine development is also hindered when natural challenge is the only means of validation. In this regard, the resurrection of "leishmanisation" sponsored by the World Health Organization and the Special Programme for Research and Training in Tropical Diseases (TDR) is a major step forward. Leishmanisation (active infection to produce natural resistance) was once common in the Middle East and eastern Europe to minimise the impact of scarring but was largely discontinued because of unacceptable lesions in some recipients. However, scientists in Iran have now produced *L major* stabilates (populations stored in a genetically stable and viable condition) to GLP standard, which should produce consistent and acceptable lesions. Leishmanisation may thus provide an ethically acceptable means of live vaccine challenge in endemic areas.

For visceral leishmaniasis, the situation remains less promising, and, although there are concerns about whether the same vaccine will work for all leishmaniases, human trials of vaccines against visceral leishmaniasis are likely to follow only from successful outcomes of those against cutaneous leishmaniasis, or as in the Sudan, on a compassionate basis.[27] Recent reports suggest some progress is being made in vaccines for canine visceral leishmaniasis.[28 w30]

Competing interests: CRD has been reimbursed by InterVet, manufacturer of Scalibor, for attending conferences. SLC was paid by Zentaris, manufacturer of miltefosine, for writing part of the dossier for drug registration.

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Lesson of the week

Fatal dysnatraemia caused by elective colonoscopy

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Colonoscopy can cause fatal dysnatraemia, and plasma sodium should always be checked after the procedure

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Recent data suggest that colonoscopy is superior to other screening procedures for the detection of colorectal cancer in people aged over 50.[1] The American College of Gastroenterology has recently recommended that individuals over 50 at average risk of colon cancer should have elective colonoscopy every 10 years, and those at higher risk more frequently.[2] In the United States, 239 000 inpatient colonoscopies were performed in 1995,[3] and this figure rose by 20% to 286 000 by 1999.[4] Yet complications of elective colonoscopy are reported to be infrequent: colon perforation or bleeding occurs in fewer than 1% of cases, and electrolyte disorders are not even mentioned.[1 5–8]

Preparation of the colon for colonoscopy involves a thorough cleansing of the large bowel by one of several different methods, in some of which large volumes of a liquid cleansing agent may be given: one method involves drinking 4 l polyethylene glycol solution; another involves taking 90 ml sodium phosphate solution.[5 9] Both methods can lead to diarrhoea with nausea, vomiting, and potential dehydration,[9] often resulting in raised plasma concentrations of antidiuretic hormone.[10] Thus electrolyte imbalance may occur, either from increased oral water intake with abnormal fluid retention or from increased fluid losses into the gastrointestinal tract.

Furthermore, preparation for colonoscopy causes substantial release of antidiuretic hormone,[10] and gastrointestinal fluid losses may cause excessive thirst, so increasing fluid intake. In patients with impaired ability to excrete water, the raised plasma antidiuretic hormone concentrations can lead to hyponatraemia; if thirst is impaired, excessive fluid losses can lead to hypernatraemia. In elderly patients in hospital, acute hypernatraemia and hyponatraemia may be fatal,[11 12] yet there are no reports of fatal electrolyte complications associated with elective colonoscopy. We recently saw three patients who developed symptoms of hypernatraemia or hyponatraemia (dysnatraemia) as a complication of elective colonoscopy.

Case reports

Over a period of 28 months (December 1998 to March 2001), JAC and AIA advised elective colonoscopy for three patients: one for unexplained weight loss, one for bleeding, and one as routine screening. Their ages ranged from 51 to 73; two were men and one was a woman. All three patients developed symptoms of dysnatraemia, and the laboratory findings at the time this was noted are shown in the table. All three patients had been given 4 l of a standard bowel preparation solution containing an isosmotic solution of polyethylene glycol and balanced electrolytes (Golytely; Braintree Laboratories, Braintree, MA) to prepare the bowel.[9 13] Plasma sodium was measured at the time patients first showed symptoms of dysnatraemia.

In patients 1 and 2, taking the cleansing solution induced nausea, abdominal distension, and diarrhoea, and both patients reported then drinking substantially increased amounts of fluids. Patient 1 was concomitantly taking thiazides. Before preparation, her plasma sodium concentration was 138 mmol/l. After drinking all 4 l of the preparation solution, she continued to drink water and reported nausea, vomiting, and headache. The following morning she was found unconscious in bed; she had several tonic-clonic seizures on the way to hospital in the ambulance; and in hospital