

Emerging parasitic infections

On a cold November evening more than 70 people attended a talk on Emerging parasitic infections, organised by the London bacteriology discussion group and held in the Prideaux Lecture Theatre at St Thomas' Hospital.

The speaker, John Williams – senior principal clinical scientist and head of the teaching and diagnostic unit at the London School of Hygiene and Tropical Medicine (LSHTM), and honorary clinical scientist at the PHLS Malaria Reference Laboratory – had a particular interest in the possible role of *Blastocystis hominis* and *Dientamoeba fragilis* in chronic bowel disease; however, in his talk on the wider aspects of parasitic infection, John stated that the parasitic infections now all appear to have emerged, and that *Giardia lamblia/intestinalis* had been recognised for the past 30 to 40 years.

Cryptosporidium parvum was the first of the intestinal coccidia to emerge as a human pathogen, being seen at LSHTM in 1975, and, although well known in veterinary circles, was not recognised as a human pathogen and initially caused diagnostic problems for routine laboratories. *C. parvum* was first described in mice in 1907 and then in humans in 1976. Laboratories that use acid-fast stains now diagnose it readily. This extra-cytoplasmic parasite is excreted as an

infective oocyst into water, from which it can cause a self-limiting infection in an immunocompetent host and a severe infection in an immunosuppressed one. Prior to the introduction of highly active anti-retroviral therapy (HAART), *C. parvum* infection could be life threatening in HIV patients, when 10 to 14 L of fluid could be lost per day. Molecular biology has advanced the understanding of the disease and confirmed that *C. parvum* has two genotypes. Type 1 is found only in man and causes endemics, whereas type 2 is found in cattle and sheep and is responsible for epidemic disease and possibly endemics.

John also described *Cyclospora cayentanensis*, which is related to *Eimeria* spp. found in birds. Occurring in the tropical and sub-tropical regions of the world – especially Peru, Nepal, Pakistan and India – this coccidian causes mild to severe watery diarrhoea and symptoms that last up to seven weeks. Most patients report an abrupt onset of mild to severe nausea, anorexia, abdominal cramps and watery diarrhoea, with weight loss occurring in five to 10 per cent of cases. Patients commonly report alternating diarrhoea and constipation.

The parasite is found in the jejunum and oocysts are passed in the faeces. It sporulates in the environment and remains infective for up to 12-15 weeks. Cysts are variably acid-fast, lose the ability to retain the Ziehl-Neelsen stain after formalin treatment, and autofluo-

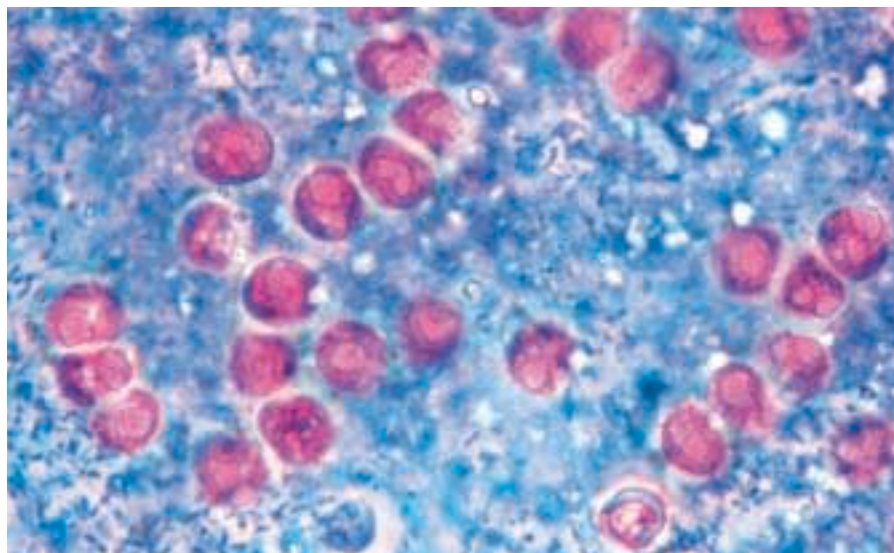
resce when illuminated with short-wavelength (340 nm) light. The infection is best diagnosed by examination of faecal concentrates or wet preparations, in which they exclude iodine; however, using molecular techniques, *C. cayentanensis* cross-reacts with *Eimeria* spp. In concluding his comments on this parasite, John was unable to explain why the expected rise in the incidence of this infection had not occurred.

The talk continued with a description of the microsporidia, of which 14 species had been described thus far. The seven genera were *Enterocytozoon* sp., *Encephalitozoon* sp., *Vittaforma* sp., *Nosema* sp., *Brachiola* sp., *Pleistophora* sp. and *Trachipleistophora* sp.; however, the life cycle, epidemiology and reservoirs of infection remain unknown.

Pleistophora and *Trachipleistophora* species have been reported in three HIV cases, but their significance in the immunocompetent host is unclear. *Enterocytozoon* sp. is seen most commonly in AIDS patients, and human-to-human transmission is suspected. *Encephalitozoon cuniculi* is rarely seen but causes encephalitis and renal infections. *E. bellam* disseminates to the eyes and the urinary tract, and it is suggested that it may be an inhaled infection.

A single *Nosema* sp. infection has been reported in an immunodeficient child with a fatal disseminated infection. *Pleistophora* sp. is very acid-fast and has been retrieved from muscle in three people with AIDS. *Enterocytozoon bienewisi* and *Encephalitozoon intestinalis* are responsible for 10-30% of diarrhoea in AIDS patients, causing chronic, watery, non-bloody diarrhoea with abdominal cramps in patients with CD4 counts of $\leq 0.1 \times 10^9/L$ that resolves rapidly when the CD4 count recovers.

The microsporidia have also been associated with sclerosing cholangitis, peritonitis and hepatitis; *E. bellam* and *E. intestinalis* have been associated with ocular and nasal infections, bronchiolitis and renal infections; and *Pleistophora* sp. has been associated with myositis. Although transmission electron microscopy (TEM) is the 'gold standard' for speciation and is used on biopsy samples, John said that standard laboratory diagnosis is made using a strong trichrome stain on a very thin smear; however, optical brighteners, Giemsa stain and serology also can be used to identify microsporidia. *Enterocytozoon bienewisi*



Cryptosporidium parvum oocysts (pink) in a stool sample. The parasite causes severe diarrhoea and is found commonly in AIDS patients

cannot be cultured and diagnosis is made on specimens of faeces and brush border lavage.

Microsporidia cause infection via a tubule that is formed on contact with a cell membrane, through which the sporoplasm is released into the cell. In HIV, horizontal transmission occurs in the gut. Systemic *Encephalitozoon bellam* infection can be treated with albendazole.

Acanthamoeba sp. infections are diagnosed in the Free-Living Amoeba Reference Laboratory and John said that referral of specimens to this laboratory had increased suddenly in 2001; however, he was uncertain about whether this was because more people were being infected or because of greater awareness of the infection. The organisms encyst on drying and excyst when favourable conditions are encountered. Positive specimens are identified using *Escherichia coli*-seeded plates.

Blastocystis hominis cysts measure 10 µm and can be grown in culture at LSHTM. In patients diagnosed with chronic irritable bowel syndrome (IBS), faecal samples are placed directly into fixative and then stained with an iron haematoxylin. Using this technique, 40% of IBS patients were shown to be infected with *B. hominis*. John then reported a small study in which <1% of faecal samples submitted for culture (ie not IBS patients) were shown to contain cysts, and symptoms disappeared after treatment with metronidazole or a corticosteroid. John said that there was no funding available for research into the link with IBS; however, *D. fragilis* is, he said, the more likely pathogen and in the UK is found in 20% of patients diagnosed with IBS.

John Williams concluded his talk by saying that emerging and re-emerging infections were important public health issues. Emergence was due to the increased number of immunocompromised patients who survive for longer periods of time. In addition, there also has been an increase in the pollution and contamination of waterways, and these act as important reservoirs for these pathogens. The ease of foreign travel and an increased awareness of parasitic disease among healthcare workers have also led to an increase in the diagnosis of pathogens in the UK.

Finally, John left the audience with one thought to ponder. No one was aware of *C. cayetanensis* in humans until the late 1980s; however, a teaching slide prepared in the 1970s and used to demonstrate *Entamoeba histolytica/dispar* cysts showed the presence of *C. cayetanensis*!.

Katherine Bowers

The meeting was sponsored by TCS Biosciences.

Breakthrough claimed in rheumatoid arthritis treatment

Side effects from drugs used to treat disease often prove more painful and problematic than the ailment that the drugs were designed to ease. This is especially true in the case of rheumatoid arthritis, a condition that affects twice as many women as it does men, usually in the over 25 age group, although sometimes the most severe form is found in children. Typically, it is treated with a steroid but long-term administration can be associated with the development of Cushing's syndrome and patients can suffer from thinning of the skin, muscle weakness and brittle bones.

Dr Declan Naughton, from the School of Pharmacy and Biomolecular Sciences at the University of Brighton, has spent 10 years researching rheumatoid arthritis and recently made a breakthrough when he demonstrated that arthritic joints have a low oxygen content and then found a way of overcoming the serious side effects associated with treatment.

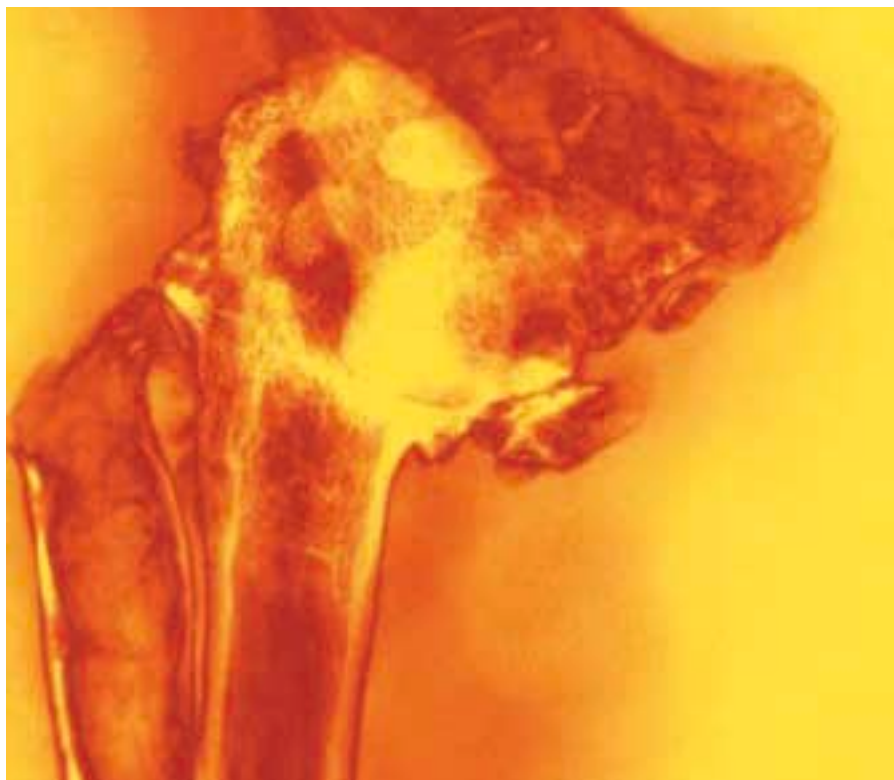
Now, Dr Naughton has found a way to ensure that the drug targets the affected tissue and not the whole body. By chemically attaching an anti-arthritic drug to

vitamin E in the laboratory, he found that the vitamin 'envelopes' the drug and prevents it from working until it comes across tissue with a low oxygen content – the arthritic joint – where the drug is released.

"It is the most exciting finding I've made in all the years I've been studying the arthritic joint," said Dr Naughton. "Since the Nobel Prize was awarded 51 years ago for the discovery of steroids and their use to treat rheumatoid arthritis, the race has been on to overcome the related serious side effects. The use of low oxygen levels in the joint for vitamin-based drug targeting is a real opportunity to bypass the side effects that currently limit the use of these amazing drugs."

Dr Naughton has published his work in a recent edition of the international journal *Advanced Drug Delivery Reviews*, and, with a patent pending on his findings, a new company is being established to take this work forward and develop it.

More details about the research are available from Dr Declan Naughton, email D.P.Naughton@bton.ac.uk



X-ray showing dislocation and destruction of the human elbow joint due to severe rheumatoid arthritis