A Fatal Outbreak of *Campylobacter jejuni* Enteritis in a Colony of Vervet Monkeys in Kenya

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Summary

In a group of 50 wild-caught vervet monkeys trapped for experimental studies, 23 developed severe diarrhoea during the quarantine period. While 10 of these responded well to routine treatment with metronidazole, kaomycin and oral electrolytes, 13 initially showed slight improvement but later relapsed. Five of these failed to respond altogether and were euthanised. Fresh faecal samples were collected from the surviving eight monkeys and analysed for microbiology and drug sensitivity. *Campylobacter jejuni*, sensitive to erythromycin, was isolated from all the faecal samples. Following treatment with erythromycin, seven monkeys recovered fully within ten days but one died before the end of therapy. This study indicates that wild non-human primates may play a significant role as a reservoir of *C. jejuni*, whereby they may act as natural carriers of this human pathogen. Screening for *Campylobacter* sp in newly acquired monkeys is advisable as part of the quarantine procedures.

Introduction

Campylobacter is the leading cause of bacterial diarrhoea in the developed world, presenting a significant challenge to public health (*Konkel et al, 2001*). Due to its ubiquitous nature, *C. jejuni* is the most common species associated with human illness and accounts for 95% of all clinical isolates in the UK (*Matsuda and Moore, 2004*). In tropical developing countries, *Campylobacter* infections are hyperendemic among young children, especially those aged <2 years. Asymptomatic infections occur commonly in both children and adults, whereas, in developed countries, asymptomatic *Campylobacter* infections are unusual (*Islam et al, 2005*). The true public health incidence, due to under-reporting, is estimated to be up to 10 times higher than documented case numbers (*Allos, 2001*). Consequently, campylobacteriosis causes substantial annual losses because of clinical costs and lost working hours (*Forsythe, 2000*). Although campylobacteriosis is self-limiting with a majority of the patients requiring no more than supportive therapy, the increasing proportion of *Campylobacter* strains that have been found to be drug resistant could compromise and prolong treatment of patients with bacteraemia. This is especially critical in developing countries where usage of antibiotics in humans and animals is relatively unrestricted (*Fields and Swerdlow, 1999; Allos, 2001*). The disease is associated with high mortality in immunocompromised patients especially those with AIDS. The most notable complication of *C. jejuni* infections is the development of Guillain-Barre syndrome (GBS), an acute demyelinating polyneuropathy (*Konkel et al, 2001*). Development

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of GBS follows gastrointestinal disease and is characterized by flaccid paralysis.

Campylobacter infection in non-human primates closely mimics the disease and immune response seen in humans (*Islam, 2005*), and *C. jejuni* mediated diarrhoea in non-human primates, responds to treatment with erythromycin (*Tribe et al, 1979; Morton et al, 1983; Bryant et al, 1983*).

In the present investigation, a batch of 50 vervet monkeys was received at Kenya Trypanosomiasis Research Institute (KETRI): of these 23 showed diarrhoea. Ten responded to the common therapy with general antibiotics and emollients, but 13 failed to respond. This investigation was therefore designed to isolate and identify the causative organism(s), to assess the associated lesions, and to determine the drug sensitivity profile of the isolated organism(s) in order to apply appropriate treatment.

Materials and Methods

Animals

Fifty vervet monkeys weighing 2.0 - 4.5 kg were trapped at Kibwezi, approximately 200 km from Nairobi, for experimental studies at the KETRI Primate Unit. The animals were housed singly in stainless steel cages in the quarantine facility. The cages were placed in such a way that the animals had visual contact with each other. They were fed two rations daily (morning and afternoon) of commercial monkey pellets (Unga Feeds Kenya Ltd, Nakuru, Kenya), fresh fruits and vegetables. Water was provided *ad libitum* in a bottle with a stainless steel ball-nozzle. At the initiation of quarantine, the animals were allowed to settle down without any laboratory procedures for the first two weeks. A screen to prevent direct eye contact with humans was placed on the front of each cage. The animals were tested for tuberculosis using the mammalian tuberculin and serum was tested for various pathogens including simian immunodeficiency virus (SIV). At the same time the animals were examined for skin lesions and ectoparasites and, when warranted, treated accordingly.

Clinical presentation and outcome of treatment

Twenty-three of these 50 vervets were noted to have persistent mucoid blood-tinged diarrhoea, which commenced on the third day of quarantine. The affected animals were in poor body condition, anorexic, dehydrated and lethargic. The peri-anal region was soiled with bloody and often mucoid faecal material matting the hair. Most of the severely dehydrated monkeys had subnormal (i.e. <35ºC) rectal temperature. They were immediately given supportive therapy consisting of oral rehydration fluids (Lectade®, Unga Feeds Kenya Ltd), oral antibiotics: 4 ml neomycin/kaolin (Kaomycin®, Upjohn, Belgium) equivalent to 7 mg neomycin sulphate, 197 mg kaolin and 4.4 mg pectin, appetite stimulants multivitamins (Parentrovite®, Bencard Brentford, England). Additionally, the monkeys were treated for intestinal protozoan parasites with metronidazole (Flagyl®, Janssen, Denmark) and helminths with albendazole (Valbazen®, Ciba-Geigy, Switzerland) following laboratory examination of faecal samples. Only 10 of the 23 monkeys recovered satisfactorily from the gastroenteritis. Five of the 23 vervets deteriorated rapidly and were euthanised by an overdose of 20% pentobarbitone sodium (Euthatal®, Rhone Merieux). Post mortem examinations were conducted and tissue samples from the gastrointestinal tract collected and processed for histopathology.

When no improvements were observed in the other eight monkeys after three days of medication, fresh faecal samples were collected from these through rectal swabs using sterile cotton swabs for microbiology. This was done when the animals were under sedation with ketamine hydrochloride at 10 mg/kg injected intramuscularly. The samples were transported to the microbiology laboratory in Cary-Blair transport media and analysed for bacteriology and drug sensitivity of isolated organisms at The Wellcome Trust Laboratories, based at the National Public Health Laboratories, Kenyatta National Hospital, Nairobi. Briefly, two samples of about 1g each were transferred to liquid enrichment medium (LEM) or directly onto charcoal-selective media

(CSM) and incubated at 42 °C in a microaerobic atmosphere (5% O_2, 10% CO_2 and 85% N_2) for 24 and 48 hours, respectively. Cultures in LEM were subcultured to CSM and incubated as described above. Colonies suspected of being *Campylobacter* sp were Gram-stained and tested for oxidase, catalase and hippurate hydrolysis.

Results

Bacteriology and Outcome of therapy

C. jejuni was isolated from the fecal samples of all eight vervet monkeys. The organism was sensitive to erythromycin and the animals were immediately put on therapy. Seven of the vervets recovered fully after treatment with erythromycin ethylsuccinate (10 mg/kg body weight, orally, for seven days), while one animal died on the fourth day of treatment.

Post mortem results

The mucosal surface of the gastrointestinal tract of the five euthanasied monkeys was severely haemorrhagic, especially along the ileum and lower gut through to the colon. There was mucosal congestion and petechial to severe haemorrhages affecting the stomach and small and large intestinal mucosa. Histopathology revealed erosion of the epithelial layer, atrophy of villi and haemorrhages in the submucosal layer. The goblet cells were distended with mucous while the gastric, pyloric and duodenal mucosal glands were hypertrophied. There was diffuse plasma cell and lymphocyte infiltration in the lamina propria and the glandular layer of the gastrointestinal tract.

Discussion

The inability to satisfy the demand for captive-bred non-human primates for biomedical research (*Hau and Schapiro, 2006*) results in the use of captive wild non-human primates in various countries including Kenya. KETRI uses wild-caught vervet monkeys (*Chlorocebus aethiops*, syn. *Cercopithecus aethiops*) as an induced model of human African trypanosomiasis (*Schimdt and Sayer, 1982; Gichuki and Brun, 1999; Farah et al, 2005*). Upon arrival, the monkeys are isolated in quarantine for 90 days, during which they are accustomed to handling and laboratory conditions and screened for various pathogens including zoonoses such as tuberculosis, simian immunodeficiency virus, and internal and external parasites. One of the most common health problems observed during quarantine is diarrhoea. This is probably associated with translocation and captivity stress and normally disappears soon after the animals acclimatize to captivity (*Tribe et al, 1979; Suleman et al, 2004*). The diarrhoea may range from watery to mucoid to bloody or a combination of any of these three faecal consistencies. Previous experience has demonstrated that the most common cause(s) of diarrhoea at the initiation of quarantine was *Entamoeba histolytica*, *Shigella* sp, *Salmonella* sp, or helminths, either singly or in combination (*Munene et al, 1998*). Treatment with a combination of metronidazole, kaomycin, oral electrolytes and anthelmintics was generally sufficient to cure the diarrhoea in newly acquired monkeys (*Munene et al, 1998*).

The findings of the present study indicate that *C. jejuni* can cause severe, and sometimes despite treatment fatal, gastroenteritis in Vervet monkeys. *C. jejuni* is a common cause of gastroenteritis in children and adults, and is frequently being identified and isolated from monkeys diagnosed with diarrhoea (*Morton et al, 1983; Bryant et al, 1983*). The pathophysiology of *C. jejuni* enteritis is unclear but involves release of enterotoxin, mucosal invasion, proliferation and release of cytotoxins. The organisms penetrate the mucosa and proliferate in the lamina propria from where they are taken up by resident macrophages, which transport them to regional lymph nodes, producing mesenteric lymphadenitis (*van Vliet and Ketley, 2001*). These modes of action may operate singly or in combination and determine the clinical outcome of the disease (*Walker et al, 1986*).

The epidemiological aspects of campylobacter enteritis and the actual role of the organism in the

production of diarrhoea have not been clearly investigated. Morton and co-workers (*1983*) reported that trapped monkeys from a non-urban, non-agricultural environment have a low prevalence of infection, implying that *C. jejuni* enteritis may not be a naturally occurring disease in the wild. They therefore suggested that monkeys become infected during the course of trapping and translocation, establishing a reservoir in captive non-human primates. While this may be the case, there is also the possibility that monkeys in the wild may act as asymptomatic carriers as has been reported for domestic cats (*Gifford et al, 1985*) and dogs (*Hosie et al, 1979*). When immunosuppressed, such carriers may later develop the disease when subjected to e.g. captivity stress. It has been claimed that birds form the main natural carriers of *Campylobacter* sp (*Skirrow, 1982*). Their droppings may contaminate the environment of the monkeys resulting in either disease or carrier status of them. The intestinal pathology reported in infected monkeys is similar to *C. jejuni* pathology in the human and in piglets (*Babakhani et al, 1993; Allos and Blaser, 1995*). However, lesions in the stomach that were prominent in monkeys are not common in other species. *C. jejuni* mainly proliferates in the intestinal epithelium of jejunum, ileum and colon leading to cellular degeneration and production of exudates into the intestinal lumen. Necrosis of epithelial cells is primarily caused by one or more bacterial toxins. The distension and hypertrophy of the goblet cells could also be due to the effect of cytolethal distending toxin produced by the bacteria (*Konkel et al, 2001*). In some cases, as in the vervets investigated in our study, there is deeper tissue involvement resulting in hemorrhagic necrosis in the lamina propria and influx of inflammatory cell exudates. Recent information on *Campylobacter* sp in Kenyan monkeys is lacking. However, Tribe and co-workers (*1979*) reported that eleven out of twelve diarrhoeic baboons imported into USA from Kenya had thermophilic *Campylobacter* sp in their faeces and the present findings indicate that monkeys may play a significant role in the dynamics of *C. jejuni* enteritis in Kenya. This stresses the need for early isolation and identification of the primary pathogen in non-human primate diarrhoea, which should be followed by sensitivity testing. This may enable early appropriate treatment reducing the risk of spreading the disease to other animals within the colony and to the laboratory staff. It is therefore recommended that screening newly acquired vervet monkeys for *Campylobacter* sp be made a routine procedure as recommended by the Federation of European Laboratory Animal Science Associations (*FELASA, 1999*). It is possible that the initial clinical improvement of the animals in our study was due to successful treatment with metronidazole and anthelmintics to clear most the sensitive internal parasites. This might have allowed the proliferation of the resident *C. jejuni* and the resurgence of the bloody diarrhoea. That erythromycin is an effective antibiotic for treatment of *C. jejuni* infections was manifested in our study where seven out of eight monkeys got cured. In infected humans, the drug is able to rapidly eradicate *C. jejuni* from the stools within 3 days.

Recent reports indicate that campylobacteriosis continues to pose a major public health problem worldwide (*Griffiths and Park, 1990; Snelling et al, 2005*) with increased incidence of antimicrobial resistance (*Fields and Swerdlow, 1999; Allos 2001; Hakanen et al, 2003*). The unravelling of these problem areas will require the development of new therapeutic agents and the vervet monkey may well provide a good animal model for testing new therapeutic agents prior to clinical testing. The model may also be useful in elucidating *C. jejuni* virulence determinants and the stages at which they contribute to infection and disease.

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