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A neglected disease of humans: a new focus of visceral leishmaniasis in Bakool, Somalia

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Abstract

Visceral leishmaniasis (VL) was observed in children in Bakool region, Somalia, an area where VL has not been reported before. We describe the extent of the problem in this war- and famine-stricken area. A retrospective analysis was done of all cases admitted to a VL treatment centre between July 2000 and August 2001. Patients with longstanding fever, splenomegaly and a positive direct agglutination test (DAT; titre > 1:3200) were treated as suspected VL cases. A rapid epidemiological and entomological assessment was performed in the area. Species identification was attempted from blood samples by polymerase chain reaction–restriction fragment length polymorphism analysis of cysteine proteinase B genes. In 1 year, 230 serologically-positive cases were diagnosed as VL, and response to therapy was good in 91.6% of the 225 treated with sodium stibogluconate. Parasitological confirmation was attempted and obtained in 2 cases. Parasites were found to be most similar to Sudanese and Ethiopian reference strains of the *Leishmania donovani* complex. In a serological survey of 161 healthy displaced persons, 15% were positive by the leishmanin skin test and 3 (2%) were positive by the DAT. The sandfly captures showed *Phlebotomus martini* and *P. vansomerenae*. VL seems to be a longstanding and serious health problem in Bakool region. Food insecurity might have contributed to the emergence and detection of VL in this area.

Keywords: visceral leishmaniasis, *Leishmania donovani*, epidemiology, outbreak, Somalia

Introduction

In July 2000, a visceral leishmaniasis (VL) outbreak occurred in the north-east of Kenya and the south of Somalia as well as in the Somali refugee population (Boussery *et al*., 2001) (Fig. 1). At that time, Médecins sans Frontières (MSF) ran a feeding programme for severely malnourished children in Huddur, Somalia, in the drought-affected region of Bakool. As several malnourished children did not improve after receiving therapeutic feeding and care, first tuberculosis (TB) and later VL was suspected. Finger-prick blood samples on filter-paper from 8 non-responding children in the feeding programme were found positive by the direct agglutination test (DAT) for VL carried out at the Institute of Tropical Medicine (ITM), Antwerp, Belgium in July 2000. The relief programme in Huddur has no laboratory facilities and subsequently, arrangements were made to send filter-paper blood samples from children with suspected VL to an MSF laboratory in Kenya. Children with clinical signs and symptoms of VL who were DAT-positive were, thereafter, treated with a standard antimonial regimen and responded well.

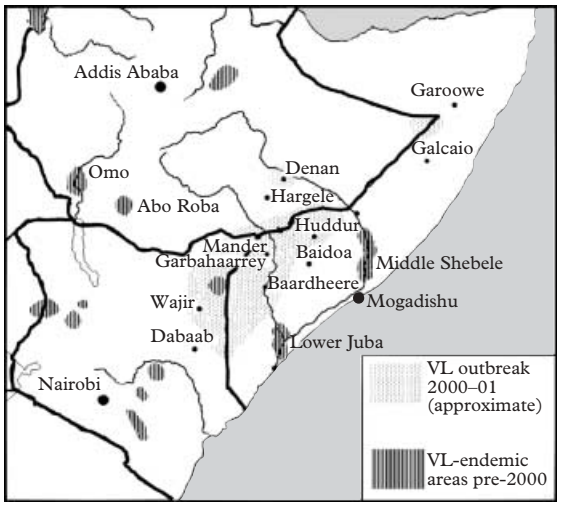

Fig. 1. Localization of recent outbreaks (2000–01) of visceral leishmaniasis (VL) and known endemic areas (pre-2000) in the Horn of Africa.

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A rapid population assessment in the Bakool area in August 2000, showed that 5% of 2221 persons examined had clinical signs and symptoms suggestive of VL, but no further confirmation was attempted at that time. The clinical syndrome of VL seemed to be familiar to the people and was perceived as an important contributor to child mortality (MSF, unpublished report). However, no case reports about VL in Bakool and Gedo regions were found in the medical literature prior to 2000. Before the civil war (ongoing since 1991), sporadic cases of VL were reported from several areas in Somalia: Penso (1934) and Moise (1955) reported VL cases from the coastal areas in the south of the country; Baruffa (1965) reported 12 parasitologically-proven cases from Middle Shebelle region; Shiddo *et al*. (1995) described a VL-endemic area along the Shebelle river in the south of Somalia; and Woolhead (1995) wrote a case report about 1 VL case diagnosed in Baidoa, Bay region, and warned of a potential increase in incidence related to the war and famine. MSF has supported the hospital in Kismayo since the early 1990s and claims that 38 VL cases were diagnosed and treated in Kismayo in 1995 and 1996. These patients came from Lower Juba region (MSF, unpublished report). We present the available clinical and epidemiological data on the VL problem in the Bakool region of Somalia, where VL has not been reported before.

Methods

We reviewed all available clinical and epidemiological data from the period July 2000 to August 2001. In the clinical case series, we included all records of DAT-positive (titre > 1:3200) clinically suspected cases admitted between July 2000 and August 2001. Between July 2000 and March 2001, any patient presenting to the MSF centre in Huddur with a history of fever for ⩾1 month and splenomegaly and/or wasting was first treated for malaria and any infection. If there was no significant improvement and the patient still

had fever at 2-week follow-up, a blood sample was taken on filter-paper to perform a DAT. If the DAT was positive, antimonial treatment was started. From the beginning of April 2001, the DAT was done at first presentation, without waiting for the result of malarial or other antimicrobial treatment. The standard treatment used was sodium stibogluconate (SSG; produced by Albert David Ltd, Calcutta, India) as a daily i.m. injection at a dosage of 20 mg/kg/bodyweight for 30 d. Cure was defined as 'no fever in the last week of the treatment, some reduction of the spleen size and improved general wellbeing'. If there were still records of fever in the last week of the 30-d regimen, the treatment was extended for 14 d.

An entomological assessment was done in March, May, June, and August 2001. Sandflies were captured at night on oily papers in the ventilation shafts of termite hills in and around Huddur. The sandflies were collected from the oily papers, washed, and preserved on the spot. They were analysed at the Ministry of Health laboratories in Nairobi, Kenya (courtesy of Dr D. Sang).

The epidemiological assessment consisted of exhaustive screening with the DAT and leishmanin skin test (LST) of all inhabitants of a camp of displaced persons from Bakool region. These people left their home areas because of deteriorating food security and had been settled for 4 years on the outskirts of Huddur town, the regional capital. As no Somalian government health authorities were available to seek ethical clearance, approval for the conduct of the survey was requested from local authorities and the MSF headquarters in Brussels, Belgium. Informed consent was obtained on an individual basis from the subject or from his/her carer, prior to enrolment. On the condition they gave informed consent, they had an LST and 1 drop of finger-prick blood was collected on Whatmann 2MM filter-paper for the DAT. The leishmanin reagent (*Leishmania infantum*, $\times 10^6$ promastigotes per millilitre, prepared and donated by Dr M. Gramiccia, Laboratorio di Parassitologia, Istituto Superiore di Sanità, Rome, Italy) was injected intradermally in a quantity of 0.1 mL into the volar part of the left forearm. The injection site was examined after 48 h and induration was read according the technique recommended by WHO (1984): by applying moderate pressure, a line was slowly drawn with a ballpoint pen about 1–2 cm from the margin of the skin reaction towards its centre. When resistance to further movement was felt, the pen was lifted from the skin. The procedure was repeated on the opposite side of the skin reaction. This procedure was repeated at right angles, to get a 2-dimensional measurement of the induration. The diameter of the induration was established by taking the mean of the distances between the 2 pairs of opposing lines. Skin reactions with an induration of $\geqslant 5$ mm were regarded as positive.

The air-dried specimens of finger-prick blood were stored in plastic bags and sent to the ITM Laboratory. The DAT was performed as reported elsewhere (Boelaert *et al*., 1999).

To identify the species, a series of blood samples (180 μL) on ethylenediaminetetraacetic acid was taken from DAT-positive patients admitted to the Huddur relief centre in 2002. Samples were mixed with the same volume of AS1-buffer (Qiagen, Hilden, Germany) and sent to ITM. DNA extraction was performed with the QIAamp DNA Blood Mini Kit according to manufacturer's instructions (Qiagen).

Characterization of parasites was performed by polymerase chain reaction–restriction fragment length polymorphism analysis of cysteine proteinase B genes (*cpb* PCR–RFLP; K. W. Quispe *et al*., unpublished data). A 1079-bp portion of the *cpb* coding region was amplified with the following *Leishmania*-specific primers: CPBFOR, 5′-CGAACTTCGAGCGCAACCT-3′ and CPBREV 5′-CAGCCCAGGACCAAAGCAA-3′. The reactions (50 μL) contained 20–50 ng DNA, 1 × final buffer, 0.5 mM MgCl2, 200 μM of deoxyribonucleoside triphosphate mix, 10 μM of each primer and 1.5 U *Taq* DNA polymerase (Eurogentec, Seraing, Belgium). Thermal cycling parameters were: (i) initial denaturation of 5 min at 95 °C, (ii) 35 cycles consisting of denaturation at 95 °C for 30 s, annealing at 53 °C for 1 min and extension at 72 °C for 1 min, followed by (iii) a final extension at 72 °C for 10 min. After ethanol precipitation of DNA, amplicons were cut with restriction enzyme *Hae*III, and patterns resolved by electrophoresis with the Bioanalyser system (Agilent Technologies, Meyrin, Switzerland): the latter system was selected for its high sensitivity and discriminatory power. DNA from the following reference strains of the *L. donovani* complex (obtained from Prof. J.-P. Dedet, Montpellier, France) was used for characterization of the field samples: MON-1, MHOM/ES/1993/PM1/LEM2608; MON-29, MHOM/FR/1996/LEM3249; MON-2, MHOM/IN/0000/DEVI/LEM138; MON-2, MHOM/IN/1996/THAK35/LEM3178; MON-30, MHOM/SD/1982/GILANI; MON-11, MHOM/FR/1980/LEM189; MON-78, MHOM/MT/1985/BUCK; and LON-42, MHOM/ET/00/HUSSEN. The uncleaved 1079-bp *cpb* amplicon was not observed with DNA of *Trypanosoma gambiense*, *Plasmodium falciparum*, and *Mycobacterium tuberculosis*. The detection threshold of our PCR was estimated to be between 10^2 and 10^3 parasites/mL blood (healthy human blood seeded with *L. donovani* promastigotes).

Results

Clinical case series

Between July 2000 and August 2001, 392 patients presented to the MSF centre in Huddur with a history of fever of at least 1 month, splenomegaly and wasting. In 59% of them (230/392) the DAT was positive, and they were treated for VL. In 2 of the latter patients, a spleen aspiration was performed, showing numerous *L. donovani* bodies (grade 5 according to WHO classification; WHO, 1996). The typical clinical presentation of VL in Huddur was a pale and wasted child aged < 10 years, with a dry cough and a swollen abdomen, and complaining of intermittent fever, poor appetite, and epistaxis (Fig. 2).

A remarkable finding from the history of the patients was the very long duration of their sickness. In the period July to December 2000, the average duration of sickness before presentation to the health facility was 19 months. This average dropped to 9 months in the period January to August 2001.

Figure 3 shows the age and gender distribution of the 230 VL patients. Only 9 of the 230 patients were aged > 15 years. The overall gender ratio was M:F = 1.58:1 ($n = 229$). In the age group 5–10 years the M:F ratio was 1.2:1 ($n = 99$).

A minority of the patients had daily fever in the observation period prior to the start of VL treatment, but most had intermittent fever. All patients had splenomegaly. The mean spleen size was 11 cm under the costal margin in the mid-clavicular line (range 3–26 cm). There was no lymphadenopathy in the patients. Pulmonary infections, including pulmonary TB, were the most common intercurrent infections. In 215 patients the weight-for-height at the beginning of the treatment was registered. In 44% of these the weight-for-height index was $\leqslant 80\%$ of the median, the cut-off for acute wasting. At the end of treatment, the average weight gain as a percentage of the weight at the start of treatment was 9.6%. The treatment regimen was well tolerated. None of the patients receiving SSG had to have their treatment stopped or interrupted because of toxic effects, though vomiting was frequent. During the study period, only 225 patients were actually started on treatment, while 5 patients died before drug treatment

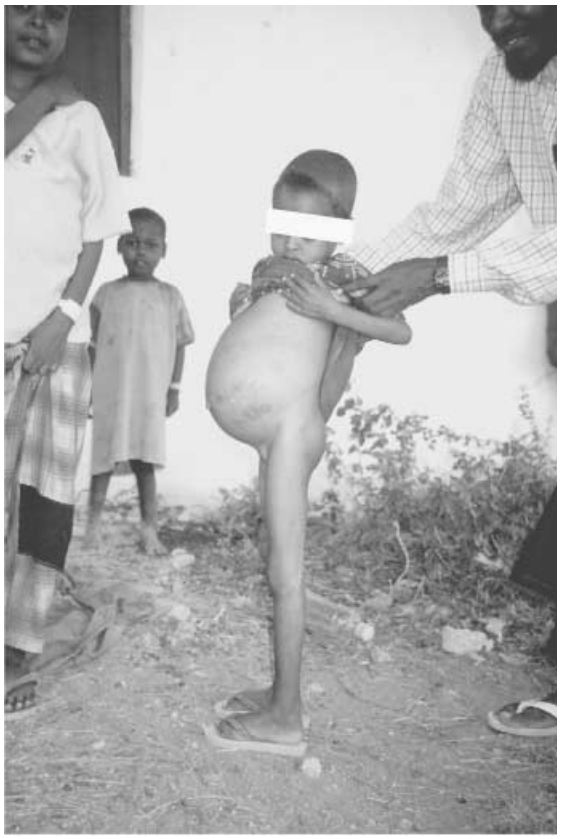

Fig. 2. Four-year-old girl with visceral leishmaniasis in Bakool region, Somalia.

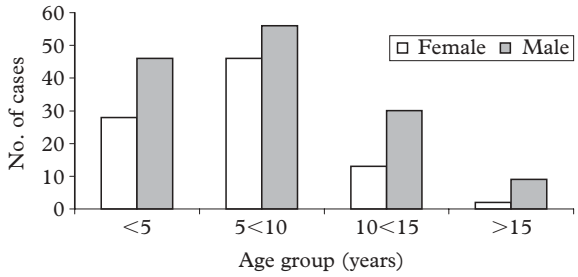

Fig. 3. Age and gender distribution of 230 visceral leishmaniasis patients admitted to a Médecins sans Frontières centre in Huddur, Bakool region, Somalia (2000–01).

could be administered. Six of the 225 patients had their treatment extended beyond 30 d. Thirteen of 225 patients (5.8%) died during the treatment. Case fatality rates significantly improved with time: in the first 6 months of the programme, the case fatality rate was 9.1% (11/121), in the next 6 months it was only 1.9% (2/104) ($\chi^2 = 5.28$, $P = 0.02$). Anaemia and its consequences were the most frequent causes of death. Spleen size at the end of the 30 d of treatment was reduced by 25–50% in 40% of the cases. In only 10% of the cases the spleen was reduced by 75–100%. Contrastingly, in the north-west of Ethiopia, 90% of treated VL patients had no palpable spleen at the end of their 30 d of treatment (M. Marlet, unpublished data).

Only 1 possible post-kala-azar dermal leishmaniasis case was recognized; a boy who did not improve very much, was still too weak to walk properly, and developed a herpes zoster at the end of his treatment. He was started on TB treatment on clinical grounds. In the first weeks of the TB treatment he developed a fine papular rash all over his body, which resolved in about 6 weeks without additional treatment. El-Hassan & Zijlstra (2001) described similar rashes. Two other patients presented again with fever and a growing spleen a few months after they had finished their primary VL treatment. On clinical grounds they were diagnosed as relapse cases. Retrospectively, these patients still showed intermittent fever in the last week of their primary treatment and they did not get the recommended extension of the VL treatment.

Entomology

The Bakool region is a rather flat area at an altitude of 550 m above sea level. The vegetation consists of many *Acacia* spp. and fewer *Balanites* spp. The rainy season of April 2001 lasted only 2 weeks. People grow maize and sorghum but also have livestock. There are large numbers of huge termite hills, spread over the fields, in the bush but also on the compounds of the people. Apart from many *Sergentomyia* spp., *Phlebotomus martini* and *P. vansomerenae* were captured from termite hills in and around Huddur.

Epidemiological screening

Figure 4 shows the age and gender distribution of the 161 displaced persons screened. Fifteen percent (26/161) had a positive LST reaction. LST positivity was significantly different according to age: only 5% (4/74) of those aged < 15 years were LST-positive, compared with 25% (22/87) of those aged $\geqslant 15$ years ($\chi^2 = 11.62$, $P < 0.001$). In the latter age group, 51.5% (17/33) of men were positive compared with 9.3% (5/54) of women ($\chi^2 = 19.36$, $P < 0.001$). Only 3 of the 161 displaced persons screened had a positive DAT; they were aged < 10 years and 1 of them had been recently treated for VL.

Parasite characterization

Parasite genotyping was performed directly (i.e. without parasite isolation) on 9 blood samples obtained from patients from Huddur. The *cpb* PCR–RFLP patterns of field samples were compared with those of reference strains representative of the genetic diversity within the *L. donovani* complex. After deduction of a few weak bands corresponding to amplification of human DNA (see open arrows in Fig. 5), patterns of field samples were found to be different from Mediterranean *L. infantum* (absence of 260-bp diagnostic fragment; see star in Fig. 5) and Indian *L. donovani* (absence of 260- and 296-bp fragments; see star in Fig. 5) reference strains, and most similar to the Sudanese reference strain of MON-30 and the Ethiopian reference strain of LON-42 (presence of 6 fragments of 71, 100, 152, 167, 186, and 217 bp; see arrows in Fig. 5).

Discussion

This is the first report of VL in the Bakool region of south-eastern Somalia. Because of the precarious health infrastructure and the weak referral capacity, spleen aspirations were not routinely done in the relief centre. However, the characteristic symptoms and signs, the serological evidence and the good response to antimonial therapy made it quite convincing that we were dealing with an outbreak of VL. The outbreak was confirmed by direct microscopical evidence of *L. donovani* in spleen aspirates obtained from 2 subjects. Furthermore, molecular characterization performed directly on blood samples from patients admitted in 2002 confirmed the presence of *Leishmania* parasites. Parasite genotyping indicated a close genetic similarity with a Sudanese reference strain classified as an ancestral zymodeme of *L. infantum* (MON-30; Rioux *et al.*, 1990) and an Ethiopian reference strain of zymodeme LON-42, classified as *L. donovani*, but phylogenetically closest to *L. infantum*. More precise typification should be confirmed by further studies, as species delineation

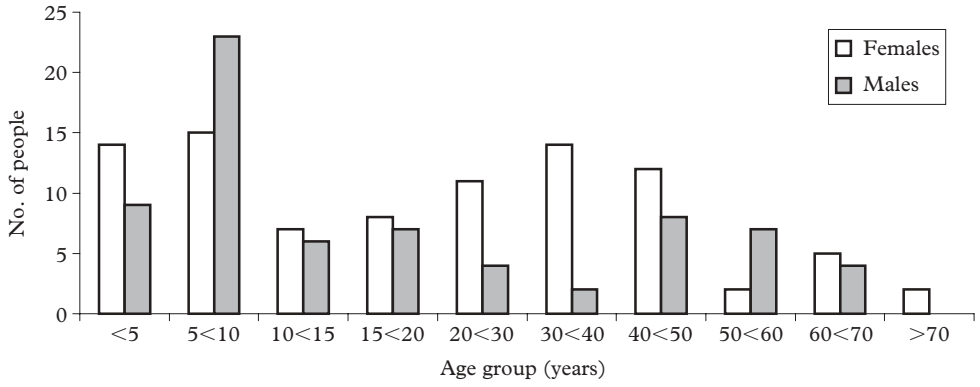

Fig. 4. Age and gender distribution of 161 displaced persons in Huddur, Bakool region, Somalia (2000–01).

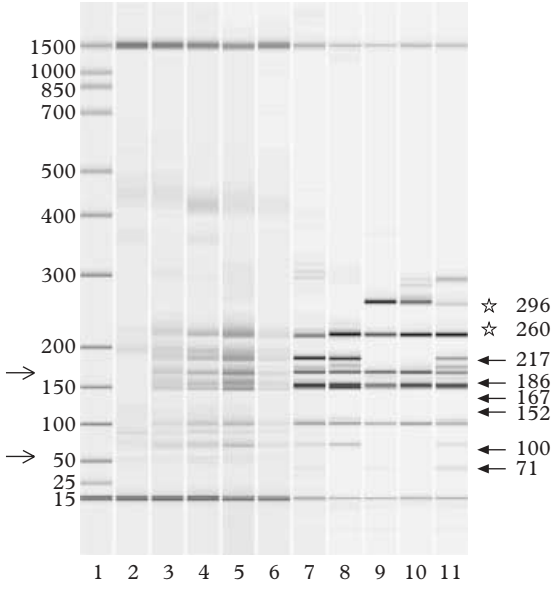

Fig. 5. Molecular characterization of samples from visceral leishmaniasis (VL) patients in Huddur compared with reference strains of the *Leishmania donovani* complex: polymerase chain reaction–restriction fragment length polymorphism analysis of cysteine proteinase B genes after cleavage with *Hae*III and electrophoresis in the bioanalyser system. Lane 1, size marker; lane 2, DNA from a healthy human; lanes 3–6, DNA obtained from blood of VL patients; lane 7, MON-30; lane 8, LON-42; lane 9, MON-78; lane 10, MON-29; lane 11, MON-2; open arrows, fragments encountered in healthy human blood and deduced from patient samples; closed arrows, 6 fragments found in East African stocks (MON-30 and LON-32) and in patient samples; stars, fragments found in *L. infantum* and Indian *L. donovani* and absent in patient samples.

within *L. donovani sensu lato* is relatively difficult in Eastern Africa, where the original focus of VL has been postulated (Pratlong *et al.*, 2001). Information obtained by qualitative methods gave no reason to believe that VL is a newly emerging problem in the area. The population of Bakool knows the syndrome of VL and calls it '*dedabsi*', meaning 'big spleen'. Traditional healers claim to have treated *dedabsi* as long as they can remember, though allegedly it may have increased somehow over the last few years. Apparently, this increase was concurrent with the drought period of 2000 and 2001. Contrastingly, in most of neighbouring Gedo, Somalia, and in Wajir district, Kenya, the population stated not to have known about VL before the outbreak of 2000–01.

The clinical presentation of VL in Bakool is also different, compared with the cases observed in Gedo and Wajir district. The cases seen in Bakool are mainly paediatric, the disease is protracted and the case fatality rate is quite low. Though our clinical case series was observed at what was originally a therapeutic feeding centre, which attracts mainly children, we are quite sure few adult VL cases escaped medical attention, as the centre was the only facility providing medical care for the whole region. This corroborates findings in the rapid population assessment carried out in August 2000. Thus, according to its paediatric profile, VL seems to be endemic in this region, in contrast to VL in its epidemic form, which affects all age ranges of a population. Children are probably exposed to the vector on or near their compounds. Both boys and girls may get infected, playing around and on top of the termite hills, the resting places of the vector. They also may get infected in the early evening or when sleeping outdoors, which is very common in this region.

We identified both *P. martini* and *P. vansomerenae* among many *Sergentomyia* spp. *Phlebotomus martini* is a proven vector of VL in other foci (Gebre-Michael & Lane, 1996) and is present throughout the year in the Konso focus in Ethiopia (Gebre-Michael & Lane, 1996). In Wajir district, Kenya and Konso, Ethiopia, *P. celiae* is also present, but mostly in the rainy season. We were not able to catch sandflies during the very short rainy season of 2001, and could thus very well have missed *P. celiae* sp. With *P. martini* being the assumed vector for Bakool, we expect the VL cases to occur throughout the year, though our observation period was too short to confirm this assumption. Also, the presented VL cases were suffering for 3 months upto 3 years. One reason for this could be that there were no health facilities in this war-stricken region over a period of at least 10 years, so cases were not treated up to July 2000.

To study the frequency of infection, we studied a purposive sample of the Bakool population: all the residents of a small camp on the edge of Huddur town, and all displaced people from other areas of Bakool region. Men aged 20–40 years were clearly underrepresented in this sample. The displaced had left their home areas mostly because of drought and famine, and this may have biased the results, because these circumstances may well have influenced both the exposure to the vector as well as the susceptibility to VL. The selection of this particular group was made for reasons of feasibility. In Somalia, insecurity and the nomadic lifestyle of the population make it very difficult to survey any community at repeated intervals of 48 h. Though not representative of the rural population in a probabilistic way, the results in displaced persons from the rural areas of Bakool show a high past infection rate, as indicated by LST positivity. Moreover, this rate is lower in young persons compared to adults. In

adults, past infection rates were significantly higher in males than in females, which might be related to the selection bias (only weakest male adults residing in the camp) or to gender-specific exposure patterns. The men work in the rather cool mornings and evenings on their fields and wander day and night through the bush with their flocks of goats and camels. Assuming that the seroconversion in DAT precedes the cellular immune response, none of the adults showed recent infection. Since their arrival in the camp 4 years ago, they were dependent on food distribution and probably reduced their activities on fields and in the bush.

VL seems to be a longstanding and serious health problem in children in this region. The age distribution of the VL cases and to a lesser extent, the results of the serological screening in displaced persons are similar to the findings of Shiddo *et al.* (1995) in Middle Shebele region. The age and gender distribution of cases and of asymptomatic individuals corresponds to that of an endemic area. Food insecurity might have increased the emergence and detection of VL in this remote and neglected area. Unfortunately, the health problems of Somalia are not on the international agenda today. Nevertheless, the health infrastructure and the food security of this population cannot be eternally neglected.

Acknowledgements

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