Operational Research in Tropical Diseases


RESULTS PORTFOLIO
Small Grants Scheme
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Operational research in the field of communicable diseases should result in cost-effective tools to achieve control, elimination and eradication. This type of research is highly relevant to communicable disease control in developing countries because it allows for better use of limited resources in providing an evidence base for health policy-making.

In the Eastern Mediterranean Region of WHO operational research that provides solutions to the problems of tropical and communicable diseases is actively promoted through the joint EMRO/DCD/TDR Small Grants Scheme, funded jointly by the UNDP/WHO/World Bank Special Programme for Research and Training in Tropical Diseases and the Regional Office for the Eastern Mediterranean. The scheme has expanded considerably since its inception in 1992, reflected in an annual increase both in the number of proposals submitted and in the number accepted for funding.

This document summarizes the outcomes of the 63 research projects implemented during the period 1992-2000. With relatively limited funds, they have all generated results that point the way in the future to practical and affordable local solutions to problems in communicable disease control, often in large contrast to the expensive and impractical measures traditionally recommended by researchers. It is a source of pride to the organizers of the scheme, as well as a measure of the quality of the research, that a total of 44 articles originated from these projects were published in peer-reviewed journals. In addition, peer-reviewed articles originating from projects funded in 2000 and 2001 will be published in a special issue of the Eastern Mediterranean Health Journal (EMHJ) for tropical diseases in 2004.

Communication of the results to policy-makers and the international scientific community through publication is considered to be one of the most important targets of the scheme. It is hoped that the results documented here will reach and influence the practice and policy-making of a wide range of health personnel in this Region.

Hussein A. Gezairy MD, FRCS
Regional Director for the Eastern Mediterranean
It has long been suggested that success in combating communicable diseases depends upon each country having the ability to conduct appropriate operational research. Operational research, which has as a prime aim the provision of interventions, tools and strategies that enhance programme effectiveness, increases the likelihood that research-derived recommendations will successfully influence policy and practice of local control programmes. Public health interventions studied in the setting in which they will be applied are more likely to take account of the vagaries of local disease epidemiology and available material and human resources. Local studies are also ideally suited to considerations of context (biological, political, socioeconomic and technological), a key determinant of the success of communicable disease control and eradication strategies. Moreover, analysis of local problems should generate locally appropriate solutions consistent with available resources.

The relevance of operational research is assured by forging a close link between researchers and local control programme management, or by equipping the control programme to conduct its own research. Therefore, capacity building is an integral part of any operational research programme.

WHO's Regional Office for the Eastern Mediterranean initiated the Small Grants Scheme for operational research in tropical diseases in 1992. The number of proposals submitted and accepted has grown constantly over the years, and especially in 2002 following expansion of the scheme to include all communicable diseases relevant to the Region. Administration of the scheme has involved coordinating research activities across the Division of Communicable Diseases, starting with translation of many disease control challenges into research priorities and ending with communication of the research results through publication, by both traditional means and electronic means with the posting of the final reports series on the EMRO website (www.emro.who.int/tdr).

Considerable disparity exists between the volume of research conducted in disease-endemic countries and the number of articles published in peer-reviewed journals. Poor skills in research methodology and in scientific writing have been the main constraints. Therefore, in recent years, the scheme has included research capacity-building in its remit, carried out through workshops on research proposal development and methodology workshops, follow-up visits, and on-line technical assistance in proposal development, data management and report writing.

A manifest benefit of this strategy is the development of a research culture within a health programme conducting operational research. Encouraging a healthy inquisitiveness in programme leaders will ensure that the right questions are posed and answered. Hence, the scheme is providing programme managers with the research design and analytical tools necessary for selecting and answering problems facing disease control in their daily practice.

Delays in publication of research results in professional journals or even exclusion owing to competition for space may effectively preclude results from influencing current policy decisions. In view of the urgent need to communicate research results to policy-makers and international scientific community, it was decided to compile and publish summaries of all the final reports submitted to the scheme during the period 1992-2000, even though some of the described strategies might have been changed over these years. This document is the product of years of dedicated effort on the part of researchers in the Region and technical staff in the Division of Communicable Diseases in their relentless fight to alleviate suffering from communicable diseases in disease endemic countries.

Dr Zuhair Hallaj
Director, Communicable Disease Control
Abstract
The aim of this study was to assess the efficiency and practicality of combined burrow destruction and removal of chenopods in the control of a zoonotic cutaneous leishmaniasis (ZCL) focus. Karameh and Sweimeh, located in the southern Jordan Valley, are stable endemic foci for ZCL. Control measures have been initiated in Karameh and were maintained for 2 years. Sweimeh was used as the control. Fifty Psammomys obesus and 6 Meriones tristami were captured from various colonies. Chenopods were uprooted and burrows destroyed to a depth of 0.5 m to 1 m within a perimeter of 2 km from Karameh. However, man-made changes to the topography have made the application of control measures more difficult. The effect of control was assessed through the comparison of leishmanin skin test (LST) positivity in children below the age of 6 years, from both foci, prior and post control. There was a significant reduction in the frequency of LST positivity after the application of control measures from 19.9% to 4.4% in the test focus in children aged 3 months to 2 years, compared to a nonsignificant reduction of 51.1% to 41.9% in the control focus. It was concluded that control measures by destruction of burrows were effective in protecting communities from contracting ZCL due to Leishmania major.

Publications

Background
Zoonotic cutaneous leishmaniasis (ZCL) is the most prevalent and only epidemiologically defined form of leishmaniasis in Jordan. The arid, semi-arid biotope of ZCL, characterized by plants of the family Chenopodiaceae, covers around 75% of Jordan's territory, however, its endemicity varies in different locations depending on ecological, environmental and socio-economic factors. The southern Jordan Valley is well known for its hyperendemicity for ZCL. Leishmania major was isolated and characterized from both human cases and Psammomys obesus. Phlebotomus papatasi was incriminated as the vector of ZCL in Jordan. In view of the high endemicity of ZCL in certain areas of Jordan and the limited application of control measures, the present study was carried out to assess the efficiency of control measures, through the destruction of rodent burrows in combination with the clearance of chenopods, on minimizing the effect of ZCL in a contained area.

Materials and methods
Karameh and Sweimeh, in the southern Jordan Valley, were chosen as the study focus. Fifty Psammomys obesus and 6 Meriones tristami were captured from various colonies. Chenopods were uprooted and burrows destroyed to a depth of 0.5 m to 1 m within a perimeter of 2 km from Karameh. However, man-made changes to the topography have made the application of control measures more difficult. The effect of control was assessed through the comparison of leishmanin skin test (LST) positivity in children below the age of 6 years, from both foci, prior and post control. There was a significant reduction in the frequency of LST positivity after the application of control measures from 19.9% to 4.4% in the test focus in children aged 3 months to 2 years, compared to a nonsignificant reduction of 51.1% to 41.9% in the control focus. It was concluded that control measures by destruction of burrows were effective in protecting communities from contracting ZCL due to Leishmania major.

Conclusions and implications of the study
This study reported the success of control measures in protecting communities from contracting zoonotic cutaneous leishmaniasis (ZCL) due to Leishmania major by the clearance of a zone of 1.4-2 km around it from chenopods and P. obesus burrows.

The clearance of chenopods is costly and ineffective as they regrow rapidly in the absence of a deterrent. Therefore, it is not recommended as part of the control measures, which includes the destruction of burrows. The latter seems sufficient to prevent P. obesus from largely recolonizing the cleared zone. This behaviour of P. obesus to withhold from colonizing cleared zones deserves to be studied on a wider scale.

Taking into consideration the cost of Pentostam treatment, the cost of control measures is reasonable when carried out to protect a non-exposed target population.

Sustainability of control measures in endemic foci and their financial feasibility depend largely on the involvement of the community and the integration of local health services to ensure the low cost and success of these measures.
area as they fall within the same environmental and ecological zones and are stable endemic foci for ZCL. Karameh was the test focus, it has a population of 8500 and is situated east of the River Jordan. To the west of the town there is a large farming area consisting of plots of planted crops irrigated by drip pipes for most of the year. Water outlets and small hills were constructed around the plots to drain water coming from the mountains during the winter and to drain the excess irrigation water to protect the land from flooding. Numerous rodent burrows were observed near houses on the western side of the town and in the farming area, the majority being located at the top edges of the water outlets and hills surrounding the plots. The control measures were applied in an area extending 2 km to the south, north and west of Karameh. Eight of the water outlets fall within this area in the west, while the east is a dry mountainous desert where burrows are absent and chenopods are scarce and scattered. Sweimeh, the control town, has a population of 2500 and has been planted with grains.

**Passive case detection** The number of ZCL cases registered in the health centres of Karameh and Sweimeh before and after the implementation of the control measures was recorded. The physicians were informed about the purpose of the study.

**Leishmanin skin test** A randomly selected sample of preschool children under 6 years of age were screened from both foci for past exposure to *L. major*. Post-control evaluation was performed on children born during the control period (2 years), i.e. on children aged 3 months to 2 years.

**Application of control measures in Karameh** These measures consisted of the clearance of chenopods and the physical destruction of burrows. Clearing the chenopds was performed manually from water outlets and from areas inaccessible to machines. All 7 water outlets, apart from the one furthest north, were removed. The tractor removed the rest of the chenopods, as well as colonies, by ploughing. The physical destruction of burrows was performed by tractors, ploughing, and by smoking in all areas with burrows inaccessible to ploughing. Although it is known that *P. obesus* burrows to a depth of 0.5 m, the soft nature of the soil and the hilly topography of many sites permitted it to reach a depth of 1 m, which added to the difficulty in the destruction of the burrows.

**Main study findings**

**Passive case detection** A total of 90 cases of ZCL from Karameh and 3 from Sweimeh were reported following the transmission season of 1992. They were confirmed either parasitologically or by LST and were children or non-immune adults.

**Baseline survey by Leishmanin skin test** Three hundred and sixty nine out of 1793 children under 6 years of age were screened, and the percentage of positive cases accounted for 33% of children in Karameh compared to 80.2% in Sweimeh. The mapping of Karameh resulted in the identification of several species of chenopods including: *Atriplex halimus*, *Suaeda vermiculata*, *S. fruticosa*, *S. aegyptica* and *Aellenia lancifolia*. *Atriplex halimus* was widespread and grew to unnatural tree-like proportions with strong deep roots. The rest of the chenopods were abundant, forming a carpet-like cover, sometimes growing amongst crops, necessitating their clearance by hand as to not damage the crops.

Regarding the captured rodents, 50 *P. obesus* and 6 *Meriones tristrami* were identified, indicating the relative abundance of *P. obesus* in the study area compared to other species. Eleven of the *P. obesus* were mature, one had an active lesion and 2 bore scars. The lesions were located on the ears, tail and nose.

**Evaluation of control activities and their cost** The number of reported cases was reduced to 20 and 6 in 1993 and 1994, respectively, indicating the success of the control activities in reducing the disease prevalence, though transmission was still possible through sandflies coming from areas where no control activities were performed. There was a significant reduction in the frequency of LST positivity after the application of control measures from 19.9% to 4.4% in the test focus in children aged 3 months to 2 years, compared to a nonsignificant reduction of 51.1% to 41.9% in the control focus. The success of control measures was also assessed by the low percentage of leishmanin conversion in initially tested negative children in Karameh compared to Sweimeh (9.8% compared to 75.9%, respectively). Interestingly, there were no attempts by *P. obesus* to recolonize the cleared zones.

**Preliminary assessment of the cost of control measures** indicated that they could be reduced if the local health authorities provide training and supervision. In addition, LST was found to be a costly technique for the evaluation of control measures.

In conclusion, Karameh presented a focus which clearly outlines the damaging effect man-made changes of a biotope can have on increasing the level of infection with ZCL. This study also provided an opportunity to thoroughly study a stable hyperendemic area of ZCL in Jordan.

The clearance of chenopods was found to be costly and ineffective as they regrow rapidly in the absence of a deterrent. Therefore, it was not recommended as part of control measures, which includes the destruction of burrows. The latter seemed sufficient to prevent *P. obesus* from largely recolonizing the cleared one.
Environmental changes to control Leishmania major cutaneous leishmaniasis in the epidemic focus of Sidi Bouzid (Tunisia). Evaluation of the impact of the modification of the reservoir host’s biotope on the transmission of the parasite

Tunisia Sidi Bouzid

Study period: June 1992–December 1993

Small Grants Scheme (SGS) 1992 No. 21

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Abstract
This study was conducted in order to evaluate the impact of the modification of the biotope of the reservoir of zoonotic cutaneous leishmaniasis (ZCL) on the transmission of the parasite. Different control methods were applied in the intervention focus (Sidi Bouzid), and the impact of intervention on disease transmission was evaluated in comparison to a control focus (Felta). Control activities consisted of the destruction of the reservoir burrows, as well as sanitation measures to eliminate breeding places for the phlebotomine sandfly vectors.

Results
A surveillance system for ZCL was implemented through Geographic Information System (GIS) software. The intervention resulted in a significant decrease in the incidence of the disease, from 1618 ZCL cases in 1991 to 339 cases in 1993. The decrease in incidence was also observed in primary schools. The intervention also resulted in a decrease in the densities of the vector in the intervention focus in comparison to the control one. The squash blot technique, using 2 DNA probes specific to Phlebotomus papatasi and L. major, proved to be useful for the monthly follow-up of sandfly infection rates when compared to the dissection technique. There was no significant difference between the 2 foci regarding the population densities of the reservoir, Meriones shawi, as no control measures were undertaken regarding this reservoir.

Conclusion
Ecological modifications of the reservoir host’s biotope had a significant impact on disease transmission and population density of the vector. On the other hand, its impact on the reservoir could not be evaluated due to unexplained decrease in the reservoir population in both the intervention and control foci.

Multicentre Collaboration
• Pasteur Institute, Tunis
• Governorate Council of Sidi Bouzid
• Regional Division of Public Health, and Basic Healthcare Service
• Ministry of Agriculture
• Ministry of Habitat and Equipment
• Municipality and Public Health sector
• Community participation

Training
The squash blot technique using DNA probes was introduced in the Laboratory of Epidemiology and Ecology of Parasitic Diseases of the Institute Pasteur de Tunis for the monthly follow-up of sandfly infection rates.

Background
More than 40,000 cases of zoonotic cutaneous leishmaniasis (ZCL) due to Leishmania major were diagnosed in central Tunisia during the period 1983-1992. Therefore, the control of the disease was one of the most important public health priorities in the country. The Sidi Bouzid governorate was the most affected area, with more than 27,000 recorded cases. The causative agent was L. major; Phlebotomus papatasi was incriminated as the vector, and Psammomys obesus followed by Meriones shawi were the reservoir hosts of the parasite. Accordingly, the control methods based on ecological modifications of the reservoir host’s

Conclusions and implications of the study
This study reported the success of control measures in protecting communities from contracting zoonotic cutaneous leishmaniasis (ZCL) due to Leishmania major by the clearance of a zone of 2 km around an endemic focus from chenopods and P. obesus burrows. This was demonstrated by the significant reduction in the number of cases reported.

GIS is an ideal tool for identifying geographic clusters of disease and is recommended for disease surveillance in endemic countries.

The squash blot technique using two DNA probes proved to be more reliable in the follow-up of sandfly infection rates in comparison to the dissection technique.

The results of this project indicate the possible existence of an unknown reservoir such as Rattus rattus in Sidi Bouzid and the possible anthroponotic transmission of L. major.
biotope were proposed to control transmission in Sidi Bouzid. This study was conducted in order to evaluate the impact of the modification of the reservoir host’s biotope on the transmission of the parasite.

Materials and methods
Different control methods were applied in the intervention focus (Sidi Bouzid), and the impact of the intervention on disease transmission was evaluated in comparison to a control focus (Felta) where no control measures were applied.

Control activities
Control activities included the destruction of Psammomys obesus burrows by mechanical ploughing of a total area of 529 hectares in the northern periphery of Sidi Bouzid town, covering a zone of 2 km radius around the town. In order to avoid desertification, the regrowth of the chenopods and the recolonization by P. obesus of the area, different species of trees, selected because of their adaptability in poor and sandy soils, were planted in the ploughed zone. These trees included Acacia cyanophylla (625 trees planted on 1 hectare), casuarina trees (15,000 trees planted on 14 hectares); jojoba trees (9325 trees on 0.5 hectare) with its economic importance as source of oil for airplane engines were recently introduced; Eucalyptus torkota (1000 trees) was also planted, but was not successful and was replaced by more acacias.

Other control activities were the salubrity and sanitation measures that consisted of cleaning the former garbage outlet of Sidi Bouzid located within the area colonized by P. obesus and that constituted an important potential breeding place for phlebotomine sandflies.

Community participation
In order to promote the programme, the planting of the trees started on 8 November 1992, which is the Feast of the Tree Day in Tunisia. This allowed the collaboration between local authorities, project participants and the whole community. Health education sessions were also held on that day.

Main study findings
Impact of intervention
The force of infection (number of infecting bites per 1000 individuals) following each transmission season till 1991 was calculated. A new census was carried out in the control focus to update the database and locate the dwellings on aerial photographs. A surveillance system was implemented in the area’s basic health centre to collect data related to ZCL (cases, newborns, deaths, emigration and immigration from the focus). A new database program was prepared using ORACLE software, followed by its introduction in a Geographic Information System (GIS), which is an ideal tool for identifying geographic clusters of disease and analysing spatial relationships between disease and risk factors. From 1983 to 1992, the annual incidence of ZCL among susceptible people in the control focus ranged from 0.04% to 11.2%, with maximum peaks in 1985 and 1987. The force of infection varied from 0.004 to 0.024. The control focus was hypoendemic during all the studied years, except in 1985 and 1987 where it was mesoendemic. These data showed the limits of the epidemic parameters when applied on human population samples residing in different geographical areas defined by administrative levels. The introduction of the GIS allowed for the clearly distinction of areas that are hyperendemic inside the sector of Felta (the control focus).

The intervention resulted in a significant decrease in the incidence of the disease from 1618 ZCL cases in 1991 to 339 cases at the end of the study in 1993. The decrease in incidence was also observed in primary schools where an active case detection was organized in October of each year during the period 1991-1993.

Monthly measurement of population densities of P. papatasi was determined by the sticky traps method and compared in the 2 foci in 9 stations, 4 in Sidi Bouzid and 5 in Felta, during the transmission seasons in 1992 and 1993. The intervention resulted in a decrease in the densities of the vector in the intervention focus in comparison to the control one. Regarding the vector infection rates, the squash blot technique using two DNA probes specific to P. papatasi and L. major proved to be more useful in calculating the monthly follow-up sandfly infection rates compared to the dissection technique. A total of 2638 sandflies were screened by this technique. The estimation of the population size of Meriones shawi was carried out by the tracking index method based on monthly measurements of rodent traces on fixed surfaces near rodent burrows. There was no significant difference between the 2 foci regarding the population densities of this reservoir as no control measures were undertaken.

Regarding the P. obesus population, the estimation was based on monthly estimates of the number of active burrows per hectare in 5 zones of 1 hectare in each focus. The results indicate that no recolonization of the zones occurred in the ploughed areas. Surprisingly, the P. obesus populations disappeared in the two foci, an unexplained phenomenon that has been previously reported from other ZCL foci in Tunisia. The infection rates of P. obesus were determined in the 2 foci, by inoculating the parasites from rodents’ ears into BALB/c mice. There was no difference in the infection rates between the 2 foci.

Conclusions and recommendations
Ecological modifications of the reservoir host’s biotope had a significant impact on disease transmission and population density of the vector. On the other hand, its impact on the reservoir could not be evaluated due to unexplained decrease in the reservoir population in both the intervention and control foci. Two hypotheses were postulated and recommended for testing: the possible existence of an unknown reservoir such as Rattus rattus in Sidi Bouzid, and the possibility of anthroponotic transmission of L. major.
Abstract

The study was conducted in endemic localized cutaneous leishmaniasis (LCL) foci in south Tunisia namely, Dhibet and Rémada. The objectives of the study were to estimate the proportion of infected children living in an endemic zone, to estimate the proportion of new cases of LCL among immune and non-immune children before the transmission season, and to determine the incidence of subclinical infections after the transmission season. Collected information included sociodemographic variables, ecological data, spatial distribution and risk factors of LCL. Children were also subjected to clinical examination and leishmanin skin testing (LST) before and after the transmission season.

Results

There was excellent concordance between the results obtained by LST and the proliferative response of the in vitro test of cellular immunity. The proportion of positivity was 38.5% and 51.1% in 5-15 year old children and scars were detected in 76.6% and 87.5% of positive individuals in Rémada and Dhibet, respectively, in comparison with less than 25% of LST-negative individuals. Patent disease was reported in half of schoolchildren in Rémada compared to 11.3% in Dhibet.

The positivity of the test in the absence of the characteristic CL scars is suggestive of subclinical infections. In endemic zones with low parasite transmission, the population will acquire immunity over seasons with or without patent disease. In this situation, the disease will affect mainly non-immune, i.e. younger individuals and new settlers. The difference in LST results in different endemic foci could be explained by the difference in the immune reaction according to the size and number of lesions, as several infections are needed to acquire immune response to infection.

Conclusion

In endemic zones with low parasite transmission, the population will acquire immunity over seasons with or without patent disease. In this situation, the disease will affect mainly the non-immune, i.e. younger individuals and new settlers. The difference in LST results in different endemic foci could be explained by the difference in the immune reaction according to the size and number of lesions, as several infections are needed to acquire immune response to infection.

Publications


Background

Localized cutaneous leishmaniasis (LCL) in Tunisia is caused by the Leishmania major zymodeme MON25 (LON1). Field studies had revealed that asymptomatic infection may occur in endemic areas. Although the extent of this phenomenon is not fully evaluated, people without a definite history of the disease may have evidence of infection as demonstrated by a positive delayed hypersensitivity reaction assessed by the leishmanin skin test (LST). This test is used to assess the prevalence of leishmania infection in human populations. Furthermore, epidemiological studies indicate that individuals with...
previous active cutaneous leishmaniasis or subclinical infection are usually resistant to a subsequent clinical infection.

The objectives of the study were to estimate the proportion of infected children living in an endemic zone, to estimate the proportion of new cases of LCL among immune and non-immune children before the transmission season, and to determine the incidence of subclinical infections after the transmission season.

Materials and methods

The study was conducted in 3 endemic LCL foci in south Tunisia, namely: El-Guettar, Dhibet and Rémada. All children aged between 2 and 11 years were included in the study. Collected information included: sociodemographic variables, ecological data, spatial distribution and risk factors of LCL. Children were also subjected to clinical examination. After the transmission season (March 1997), another clinical examination was performed in order to determine new cases of LCL.

LST was performed by injecting 0.1 ml leishmanin in the forearm and evaluating the response 72 hours later. A study of the concordance between the LST and tests of cellular immunity (in vitro) was performed on young adults (18-20 year-olds) as well as individuals who previously tested positive by LST in an earlier study.

Main study findings

There was excellent concordance between the results obtained by LST and the proliferative response of the in vitro test of cellular immunity. A total of 595 schoolchildren aged 5-15 years were enrolled before the transmission season in Rémada and Dhibet and were examined for the LCL characteristic scars, then tested with LST. Considering induration with a diameter greater than or equal to 5 mm as a positive response, and 2 to less than 5 mm as the intermediate response (LST-interm), the proportion of positivity was 38.5% and 51.1% in Rémada and Dhibet, respectively. Positivity increased with age to reach 60% at 14 years of age.

The LCL scar was detected in 76.6% and 87.5% of positive individuals in Rémada and Dhibet, respectively, in comparison with less than 25% of LST-negative individuals. Interestingly, in the LST-interm group, a proportion midway between negative and positive individuals recorded the characteristic scar (60%).

New cases were detected during biweekly school visits. Diagnosis was confirmed by Giemsa staining and culture of the parasite on NNN medium. Half of the schoolchildren in Rémada were affected compared to 11.3% in Dhibet.

During the transmission season of 1997-1998, surveillance revealed that 76% of individuals initially LST-negative contracted LCL infection compared to 17.5% and 44% of individuals initially LST-positive and LST-interm, respectively. In Dhibet, 18% of initially LST-negative individuals contracted the infection compared to 5.2% of initially LST-positive individuals. The relative risks of contracting LCL infection in LST-positive individuals revealed a protective effect in comparison with LST-negative individuals.

The positivity of the test in the absence of the characteristic CL scars are suggestive of subclinical infections. However, minimal lesions could be healed without leaving scars. In order to test the hypothesis of the presence of subclinical infections, a second LST was performed on all individuals. In Rémada, almost all individuals who developed the disease became LST-positive, but among those who were initially LST-negative, more than 38% became LST-positive and 12.3% LST-interm, indicating that these individuals developed subclinical infections. On the other hand, those with initial LST-interm reactions developed a positive reaction with or without overt clinical disease.

By contrast, 18.2% of those initially negative became positive during the second LST screening in Dhibet. These differences between the 2 foci could be explained by a greater immune reaction in Rémada’s patients as compared to Dhibet’s patients, as the lesions in the latter tended to be more frequent, solitary and smaller in size than in the former focus. Another explanation was that several infections are needed to acquire immune response to infection and that only one will develop into patent infection mainly due to a certain critical number of the parasite. In Dhibet the proportion of subclinical infections detected was comparable to that of patent infection which exceeded the proportion detected in Rémada, indicating that in foci with low parasite transmission the proportion of subclinical infections is higher than in those with high parasite transmission. This latter observation indicates that in endemic zones with low parasite transmission, the population will acquire immunity over seasons with or without patent disease that will be mostly benign (solitary lesions and of small size) and that are frequently missed, and this immunity will be associated with resistance to reinfection during epidemics. In this situation, the disease will affect mainly non-immune, i.e. younger, individuals and new settlers.

The difference between the 2 foci was also evident concerning the diameter of induration: In Rémada the diameter of induration increased after the second LST, while in Dhibet the increase in induration was less significant in those initially negative, and almost nil in previously positive or diseased individuals.

The second LST tests revealed that, between May 1997 and 1998, 90.3% compared to 56.4% of schoolchildren in Rémada and Dhibet, respectively, became immune against LCL.
Abstract
The epidemiology of cutaneous leishmaniasis (CL) was investigated in Jericho, Jordan Valley, during the period 1994-2001. Clinically suspected patients referred during 1994-1999 were interviewed and subjected to laboratory diagnosis, and plotted on two maps for the Jordan Valley and Jericho city. This was coupled with a seroprevalence survey in the area (2000-2001).

A case-control phase was also conducted in 2000-2001 whereby the household members of patients from Jericho city and Aqbat-Jaber refugee camp (139 individuals) were compared to 108 randomly selected individuals living in their neighborhood. Study subjects were interviewed regarding the determinants of infection.

Results
More than half (52%) of suspected and referred patients were from Jericho city, followed by Aqbat-Jaber (15%), then other areas in the valley.

A case-control phase was also conducted in 2000-2001 whereby the household members of patients from Jericho city and Aqbat-Jaber refugee camp (139 individuals) were compared to 108 randomly selected individuals living in their neighborhood. Study subjects were interviewed regarding the determinants of infection.

Case mapping within the Jericho city boundaries showed clustering of cases in the south-eastern parts of its periphery. The distribution of cases in Aqbat-Jaber camp was comparable to that of the city. Transmission of infection was associated with periods of high rainfall, peaking between October to January.

During the period 2000-2001, a total of 471 suspected individuals were referred for diagnosis, out of whom 32% proved to be positive by direct smear diagnosis. These were mainly children, young Palestinian soldiers camping in Jericho's outskirts, and bedouins' and farmers' wives.

The seroprevalence of CL in Jericho city and its adjacent Aqbat-Jaber refugee camp amounted to 26.3%.

Vulnerable groups are children, young Palestinian soldiers camping in Jericho's outskirts, and bedouins' and farmers' wives. The education level of the head of the family and children sleeping unders nets were the only significant determinants of infection.

Species identification showed that Leishmania major coexists with L. tropica in the Jericho District, Jordan Valley.

Background
Jericho in the Jordan Valley is the centre of a region where cutaneous leishmaniasis (CL), or "Jericho boil", caused by Leishmania major, is endemic. CL in the Jordan Valley is a vector-borne zoonotic disease, with the sand rat, Psammomys obesus, as the animal reservoir, and sandflies, Phlebotomus papatasi, as the vector. These sandflies live in rodent burrows in very close contact with the sand rats. Humans are only incidentally infected, acting as secondary hosts in the case of L. major. However, other species, such as L. tropica, are anthroponotic, where humans are the primary hosts and reservoirs for new human infections of CL.

This study was funded in 2000 to complete an earlier study initiated in 1994, aiming to determine the prevalence of CL in this area, study its risk factors, map cases and develop a database for a District Database for the Ministry of Health.

Materials and methods
Local database (1994-1999)
Suspected referred patients were
interviewed according to a questionnaire and subjected to diagnostic tests. The questionnaire included information regarding demographic data, clinical features, risk factors, laboratory diagnosis, and treatment details. Diagnosis was performed by direct tissue smear, culture on NNN media and, on rare occasions, by histopathology.

**Case-mapping (1994-1999)** All suspected referred patients were plotted on two maps for the Jordan Valley and Jericho city.

**Seroprevalence study (2000-2001)** Blood samples were collected and the CL seroprevalence was determined using the ELISA method.

**Case-control phase (2000-2001)** Cases consisted of the household members of CL patients from Jericho city and Aqbat-Jaber refugee camp, totalling 139 individuals. The control group included 108 individuals from 18 households, who were randomly selected from both the city and the camp. Study subjects were interviewed regarding the determinants of infection such as sociodemographic factors, presence of a nearby farm, breeding places in the vicinity, air-conditioning use, types of roofs and walls of the houses, cracks in walls, window screening, presence of pets, types of trees, outdoor sleeping patterns, lifestyles such as having evening tea in yards, and children sleeping under nets.

### Main study findings

During the period 1994-1999, a total of 114 patients were diagnosed in the Jordan Valley. More than half of the patients were from Jericho city (52%), followed by Aqbat-Jaber (15%), A’uja village (13%), Ein-Assultan area (6%) and Zubaidat (5%). These areas share the common feature of neighbouring agriculturally active areas. These cases were mapped at two levels: the Jericho District level, and the city and refugee camp level. Case-mapping within the city boundaries showed clustering of cases. Thirty cases (45%) were clustered in the south-eastern parts of the city periphery. Moreover, the distribution of cases in Aqbat-Jaber camp was comparable to that of the city.

The peak of infection was in 1995 (31% of all cases), which coincided with the highest period of rainfall. Moreover, about 57% of patients were diagnosed from October to January, indicating a seasonal pattern of transmission, though some cases persisted through the whole year.

During the study period, 152, out of 471 suspected individuals referred for diagnosis, were proved to be positive by direct smear diagnosis (32%). More than half (57%) of the referred individuals were children under 15 years old, of whom 32% were infected, and 63% of the infected children were under 5 years old. There was no significant difference between males and females regarding the frequency of infection among clinically suspected individuals (32.6% and 31.7%, respectively).

The occupations of referred adults were as follows: 51% were Palestinian soldiers (20-29 years old) camping in Jericho's outskirts following the 1994 “Oslo Agreement”, while 13% were housewives, belonging to either bedouin or farming families. The serosurvey conducted in Jericho city and its adjacent Aqbat-Jaber refugee camp showed a seroprevalence of 26.3%. ELISA positive cases belonged to 3 groups: 67% of smear-positive individuals, 42.9% of suspected cases (recent and old infections), and 17% of healthy individuals (no evidence of infection).

ELISA revealed a sensitivity of 67%, a positive predictive value of 55%, a specificity of 85%, with a negative predictive value of 90%.

**Clinical features** More than half of the CL confirmed cases had single lesions, 22% had two lesions and 22% had 3 or more lesions. Lesions were mainly on the head and neck (52%), upper limbs (28%) and lower limbs (19%). Head lesions were mainly on the cheek or nose, and less frequently on the forehead; these sites are significantly at higher risk of getting CL in children than in adults.

In children, the head is more frequently (61%) the site of infection of CL than are other body sites, whereas sites of infection on adults are most frequently (78%) on the limbs. This indicates a significant association between age and the site of lesions.

**Determinants of infection** Among several potential risk factors for infection, the only significant determinants were the education of the head of the family and children sleeping under nets.

*L. tropica* in the Jericho District *L. tropica* was first isolated in the Jordan Valley in the summer of 2000 and was not previously reported in the Jericho District, Jordan Valley. Unlike *L. major*, which was restricted to the flat alluvial soil of the Jordan Valley, *L. tropica* existed in the rocky areas in the northern and southern parts of the Jericho District, Jordan Valley, with the sole exception of one isolate from the hilly areas close to Jericho city. Isolates were characterized by excreted factor (EF) serotyping and polymerase chain reaction (PCR)-based techniques.
Abstract

The dynamic of the leishmania life cycle in human-infected macrophages could explain the differences in *Leishmania tropica* and *L. major* infection in humans and could explain the reasons for non-response to treatment. This project was conducted to study the dynamic of *L. tropica* and *L. major* amastigote growth within macrophages in human cutaneous leishmaniasis.

Six groups of healthy and infected subjects with *L. major* or *L. tropica*, susceptible or resistant to treatment, were enrolled in the study. Peripheral blood mononuclear cells (PBMCs) were isolated from peripheral blood by macrophages. The cells were counted, cultured, incubated in a CO2 incubator, then exposed to metacyclic promastigotes of *L. major* or *L. tropica*. The intensity of the *in vitro* infection was determined by the number of amastigotes per 100 infected macrophage cells and the percentage of infected macrophage cells in different time-frames. The life cycle of the parasites was investigated and compared among different groups.

Results

There was no difference between *L. tropica* and *L. major* infection in human-infected macrophages. On the other hand, there was a significant difference between the susceptible and resistant groups regarding the number of infected macrophages as well as the number of amastigotes per macrophage. The number of macrophages and amastigotes were significantly higher in the resistant groups in comparison to the susceptible groups. The results also confirmed that once the parasite is established inside the host cell, it exhibits a life cycle consisting of an initial stage where the proliferation of the parasite is inhibited for the first 3 to 4 days after penetrating the host cell. Proliferation resumes on the fifth day in cultured macrophages, reaching a peak in the number of intracellular parasite on day 7 of the *in vitro* infection.

Background

Peritoneal macrophages infected *in vitro* with *L. donovani* showed that once the parasite is established inside the host cells, it exhibits a definite life cycle consisting of a stage where the proliferation of the parasite is inhibited during the first 3-4 days after penetration in the host cell, with proliferation resumed on the 5th day in the cultured macrophages, reaching a peak on day 7, with no significant difference in the number of amastigotes in resistant or susceptible peritoneal macrophages 24 hours after exposure to promastigotes [1]. Progression of the infection to initially uninvaded cells suggested that the infection of previously unpenetrated cells involved a "recruitment" into the susceptible pool of cells due to action of the soluble factor produced *in vivo* [2] and *in vitro* [3] by infected macrophages. The intensity of the activation of intramacrophages suggested that the susceptible strain harboured more parasites on day 7 than the peritoneal cells of the resistant animals. This study was conducted to investigate the dynamic of *L. tropica* and *L. major* amastigote growth within macrophages in human cutaneous leishmaniasis. The dynamic of the leishmania life cycle in human-infected macrophages could explain the differences in *L. tropica* and *L. major*. 

Conclusions and implications of the study

- There is no difference between *L. tropica* and *L. major* infection in human-infected macrophages. The only difference is between resistant or susceptible groups regarding the number of infected macrophages and the number of amastigotes per macrophage.
- The life cycle of the parasite consists of an initial dormant stage of 3-4 days after infecting the cultured macrophages followed by a proliferation stage on the fifth day, then a peak in the seventh day of the *in vitro* infection.
infection in humans and could explain the reasons for non-response to treatment.

Materials and methods
Life cycles of *L. tropica* and *L. major* were investigated in healthy and infected patients. Six groups of 5 subjects each were selected for the study: Groups 1 and 2 consisted of healthy volunteers with no history of cutaneous leishmaniasis; Groups 3 and 4 included subjects infected with *L. tropica* and *L. major*, respectively, resistant to treatment; Groups 5 and 6 included subjects infected with *L. tropica* and *L. major*, respectively, susceptible to treatment.

The blood samples were taken from the patients when they were referred to the Centre for Research and Training in Skin Diseases and Leprosy. Peripheral Blood Mononuclear Cells (PBMNs) were isolated from peripheral blood by Ficoll gradient centrifugation and the macrophages were isolated. The cells were counted by a haemocytometer and the number of viable cells was determined by trypan blue. One hundred microliter aliquots of cell suspensions (10^7 viable cells/ml) was placed on 18X 18 mm coverslips and each coverslip was placed in a well of 6-well Linbro culture plate.

The cells were incubated in CO₂ incubator for 2 hours prior to exposure, then the cells were exposed to 10 metacyclic promastigotes of *L. major* or *L. tropica* per each macrophage. The intensity of *in vitro* infection was determined by both the number of amastigotes per 100 infected macrophages and as percentage of infected macrophages during certain period of time (0,1,3,5,7,10, 14 days). The life cycle of the parasite was investigated and compared among different groups. Statistical analysis was performed in order to compare the difference in the number of infected macrophages and number of amastigotes per macrophage in time frame of infection within each group as well as between the different groups.

Main study findings
There was no significant difference in the number of infected macrophages and the number of amastigotes per macrophage, neither between the 2 healthy groups nor groups 3 and 4 resistant to treatment, nor groups 5 and 6 susceptible to treatment. On the other hand, there was a significant difference between the susceptible and resistant groups regarding the number of infected macrophages as well as the number of amastigote per macrophage. The number of macrophages and amastigotes were significantly higher in the resistant groups (3 and 4) in comparison to the susceptible groups. The results also confirmed that once the parasite is established inside the host cell, it exhibits a life cycle consisting of an initial stage, where the proliferation of the parasite is inhibited for the first 3 to 4 days after penetrating the host cell. Proliferation resumes the fifth day in cultured macrophages, reaching a peak in the number of intracellular parasite on day 7 of the *in vitro* infection.

References
Abstract
A field intervention trial to control cutaneous leishmaniasis by using pyrethrin-impregnated bednets was carried out in Aleppo, Syria. Four villages, 20 km north-east of Aleppo, were selected as the study area. Two villages were randomly chosen and were considered intervention villages and the other two were the control villages. A total of 4578 individuals in 508 households were enrolled in the study.

During May 1994, a house-to-house survey was conducted to collect census, socioeconomic, and baseline epidemiological data. Pyrethrin pre-impregnated bednets were distributed to household members of the intervention villages. Bimonthly entomological surveys of the vector Phlebotomus sergenti were conducted using oily papers in 10 selected households in the intervention and control villages. Epidemiological and entomological surveys were repeated post-intervention. Furthermore, leishmanin skin tests were carried out on children of all the villages to estimate the force of infection.

Results
There was a 45% reduction in the incidence of cutaneous leishmaniasis in the intervention area compared to a 75% increase in the control area. These encouraging results suggested a high efficacy of Pyrethrin-impregnated bednets as a method for the control of cutaneous leishmaniasis in endemic areas.

Background
Aleppo has been endemic for cutaneous leishmaniasis for 2 to 3 generations, but its incidence rose sharply in the mid-1980s and reached its peak early in the 1990s. Pyrethroid residual spraying has been used to control malaria and leishmaniasis and, in spite of the efficacy of spraying, its main disadvantages were: high cost, low sustainability, logistical and managerial problems, high rate of nonacceptability, low community participation and the need for trained personnel.

In spite of the limitations of residual spraying, in addition to the success of insecticide-impregnated bednets trials in malaria control, it was expected that successful results with anthroponotic cutaneous leishmaniasis would be obtained. Unlike zoonotic leishmaniasis, anthroponotic cutaneous leishmaniasis can be controlled by controlling the sandfly vector transmitting the disease.

This trial was conducted to evaluate the efficacy of insecticide-impregnated bednets in controlling anthroponotic cutaneous leishmaniasis in rural Aleppo, Syria.

Materials and methods
Four villages with the highest recorded prevalence of cutaneous leishmaniasis cases were selected. The main activities of the population were agricultural and industrial. Government and private poultry farms were scattered in the area. It also included a borrow-pit for the disposal of solid wastes from Aleppo city. The total population of the study area was 4578 inhabitants in 508 households, and they were mainly farmers. A pilot study was conducted during 20-26 April 1994 to

Conclusions and implications of the study
- Pyrethrin insecticide-impregnated bednets are an efficacious method for the control of cutaneous leishmaniasis in endemic areas.
- Non-acceptability of bednet usage was mainly associated with inadequate knowledge regarding the disease. Therefore, educating communities about the new intervention method proved to be very effective in motivating these communities towards its use.
- Ensuring the sustainability of usage of insecticide-impregnated bednets in endemic communities was emphasized.
provide a rough estimate of the number of cases in each village, and the results were as follows: 1.2% in Halisa, 3.3% in Tal Shaeer, 1.4% in Kaffar Sagheer, and 2.5% in Sheikh Najjar.

**Human behaviour in respect of sleeping**

Usually people wake up at 6:00-8:00 a.m. and sleep early at 20:00-22:00. In summer, all household members sleep in the courtyard. Some individuals use local bed nets. There are some ceiling fans in the bedrooms and living rooms.

**Entomological survey**

Ten households were selected in each of the intervention and control areas for the entomological survey. Oily papers were attached to nails in the four corners and the front walls in two bedrooms in each household. Two oily papers were placed in the kitchen and two in the stable or animal shelter. Six oily papers were placed in the courtyard. They were kept for 3 nights in the household to trap sandflies.

A baseline survey was conducted at the start of the project during May-June 1994 to estimate the 1-year prevalence of the disease between July 1993 and June 1994. Other information was collected including sociodemographic data, factors influencing disease transmission, clinical and parasitological data. Incidence of the disease was estimated between December 1994 and June 1995.

**Pre and re-impregnation of bed nets**

In May 1994, 800 Pyrethrin-impregnated bednets were distributed to the households in the intervention villages. These were re-impregnated in May 1995 using WHO recommended techniques. Extra 400 impregnated bed nets were distributed in May 1995. Similar number of non-impregnated bed nets were distributed to households in the control villages.

**Health education**

Villagers were educated regarding the role of bed nets in controlling the disease. The aim of the health education was to increase the use of bed nets. Visits to the study area were carried out in evening to observe the use of bed nets.

**Main study findings**

The population and households of the intervention and control villages were comparable regarding the household size, number of rooms per household, usage of local cloth bednets (11.7%), usage of fans (74.2%) and insecticide spraying of their plants in the courtyard (31.6%).

The main reasons for not using bednets were their substitution by fans, their cost, indoor sleeping, and the absence of flies, among other reasons. The incidence of cutaneous leishmaniasis in the pre-intervention infection season and in the post-intervention infection season was as follows: in Halisa and Sheikh Najjar, the intervention villages, there was a reduction in the incidence of cutaneous leishmaniasis from 4.7% to 2.4% and from 4.6% to 3.2%, respectively. On the other hand, the incidence of cutaneous leishmaniasis increased in the control villages, Tal Shaeer and Kaffar Sagheer, from 3.5% to 5.5% and from 1.6% to 3.1%, respectively. These results indicate that there was about a 45% reduction in the incidence of cutaneous leishmaniasis in the intervention area while it increased by approximately 75% in the control area.

The entomological survey demonstrated a higher density for both male and female *P. sergenti* caught in 1994 per night/m² of oily papers. Two peaks of sandfly density were identified, one in mid-July and another in October. The outdoor density was significantly higher than the indoor density.

**Conclusion**

Insecticide-impregnated bednets are an efficacious method for the control of cutaneous leishmaniasis in endemic areas.
Abstract

Local production of the direct agglutination test (DAT) is imperative to the effective control of visceral leishmaniasis (VL) in Sudan. Establishing an aseptic *in vitro* Leishmania promastigote mass culturing is experienced as a most intricate, however, crucial step in the production of a valid DAT antigen. The successful use of the indigenous *L. donovani* strain (LD43) in this study is considered indispensable for the continuity of local antigen production.

Results

In spite of the sub-optimal laboratory conditions encountered, the research group at Ahfad University for Women succeeded in the production of 3 DAT antigen batches. The amount of antigen produced was sufficient for performing 880 full-out titration tests or 3666 screening doses for positive/negative results. The locally produced antigen batches showed a high level of reproducibility versus both serum and blood samples. By comparison with the reference antigen, the local antigen revealed similar levels of sensitivity and specificity against all 48 serum samples tested. No cross-reactions were noticed versus sera from endemic or non-endemic controls nor from cases with proven malaria or onchocerciasis. The locally produced antigen also showed a desirable degree of stability at ambient temperatures (28-35 °C) for at least 42 days. The DAT results obtained through blind testing of 67 VL-suspected patients correlated positively with VL final diagnosis in 22 of these patients and further confirmed the reliability of the locally produced antigen. Large-scale DAT production was successfully achieved reaching net antigen volumes of 750, 800, 1150 and 1250 ml. DAT antigen specimens sufficient for performing 120-2000 tests were distributed to 5 institutions involved in the routine diagnosis and epidemiological studies of VL.

Activities achieved within the framework of the study

A well-equipped research laboratory was constructed in the University. To ensure the continuity and dissemination of the DAT technique, 4 post-graduates (biologists) from the Ahfad University for Women and the Institute of Endemic Diseases (University of Khartoum) were involved in all steps of antigen processing and in the execution of the test. Through the training of the biomedical staff and the establishment of facilities in Ahfad University and in the Institute of Endemic Diseases, local DAT production was achieved. This will contribute in the reduction of the costs of kala-azar diagnosis in Sudan.

Background

The imported direct agglutination test (DAT) proved to be the most effective, though costly, method in the diagnosis of kala-azar. This prompted the investigators to undertake this study aiming at establishing local production of DAT for visceral leishmaniasis, then evaluating its reliability in diagnosing the disease.

Materials and methods

Leishmania cultures were kept in locations where the ambient temperature was relatively low and stable (25-29 °C). An isolate of *Leishmania donovani*,...
designated as LD43, and kept in continuous culture using NNN or RPMI, was provided by the University of Khartoum. The strain was then kept in GLSH (glucose/lactalbumine/serum/haemin) medium. Promastigotes of LD43, cultured continuously in this medium, maintained the desired elongated spindle form and suitable rate of growth. Supplementation with gentamicin was essential to overcome the frequent bacterial contamination. Subculturing of the strain was performed every 4-7 days, before the appearance of the degenerative round forms. Gentamicin was added and the test plates were incubated at room temperature for ±20 hours. After dilution of the antigen batches produced according to the procedures described by Harith et al., 1988. The air-dried blood samples were tested by existing spots of 6 mm diameter and eluation in 760 ml saline solution for 24 hours at 4 °C. The blood eluate so obtained was found to be equivalent to 1:100 serum dilution. Visual reading of the DAT was by localizing a clear sharp-edged blue spot, identical to the one observed in the control, and the preceding dilution is considered the titre of the test serum. All test results were read by at least 2 observers.

### Main study findings

Three antigen batches, sufficient for the testing of 176-536 individuals by full-out titration or for 733-2233 screening tests, were produced. The reproducibility of the results obtained both in the serum and blood samples was excellent. Further standardization in this procedure is expected to reduce this difference in test readings between the 2 sampling methods. By comparison with the reference antigen (ITMAP-Belgium), the locally produced one evidenced an identical level of specificity (titres <= 1:100) versus clinical disorders other than the kala-azar tested.

The sensitivity of the local antigen versus the kala-azar cases tested was comparable to the reference antigen. However, in 5 sera from kala-azar cases, the locally produced antigen was more sensitive (1-4 titre dilutions higher) by comparison with the reference. This might be due to the incorporation of the autochthonous Leishmania strain (LD43) from the same endemic area (Gedarif) in antigen processing. The locally produced DAT antigen showed stability at ambient room temperatures of 28-35 °C for at least 42 days. Although no comparison was made versus the reference antigen in this respect, a desirable length of shelf-life time was adequate for maintaining antigen reactivity under prolonged electric failures and for performing field surveys without refrigeration. A modification of the staining procedures was introduced by incubating the formaldehyde-fixed promastigote with Coomassie Brilliant Blue overnight at +4 °C leading to an increase in the dye intake by ≥95%, thus resulting in an increase in the net volume of the antigen produced. The reliability of the locally produced DAT antigen was further confirmed by carrying out blind testing on 67 blood specimens sent by MSF-Holland. The results obtained correlated positively with the disclosed final VL diagnosis in 22 patients. Relative large scale production of the DAT antigen was achieved on 4 different occasions, attaining volumes of 750, 800, 1150 and 1250 ml. Various quantities of the DAT antigen were dispatched to 5 institutes at the national level to assist in VL epidemiological studies and routine diagnosis.

### Preparation of the DAT antigen

The parasites were spun down in a refrigerated (4 °C) centrifuge at 3000 g for 15 minutes. Further steps for antigen processing were done according to Harith et al, 1995. After centrifugation, the promastigote pellet was washed 3 times in Locke's solution and treated with 2-Mercaptoethanol (2-ME) instead of trypsin. A concentrated promastigote suspension of 100 ml of 1×10⁸/ml was washed by centrifugation and resuspended in a similar volume of Locke's solution to which 1.2% (v/v) 2-ME was previously added. The treated parasite suspension was incubated at 37 °C for 45 minutes. Promastigotes were then washed again 3 times in Locke's solution and fixed in the same medium, supplemented with 2% (w/v) formaldehyde. The fixed, ready-for-use antigen, so prepared, was stored at 4 °C until required. Antigen batches were produced. The reliability (sensitivity and specificity), reproducibility and shelf-life time of the batches were assessed against clinical specimens (serum and blood) from kala-azar patients and from individuals with other conditions.

### DAT execution

Sera were tested against the antigen batches produced according to the procedures described by Harith et al., 1988. After dilution of the test sera, the antigen was added and the test plates were incubated at room temperature for ±20 hours.
Abstract
The latex agglutination test (Katex) for the detection of leishmania antigen in the urine of patients with visceral leishmaniasis was studied in Gedarif State, Eastern Sudan. The aim was to test its performance as an early diagnostic tool for screening populations living in remote areas with limited accessibility to health services. Moreover, the fact that urinary antigens decline quickly after treatment was the basis for studying the prognostic potential of the test.

A prospective study was carried out in the kala-azar treatment centres in three villages during the period April 2001-February 2002, whereby urine samples from 204 patients with suspected kala-azar were tested with Katex and the results compared to smears of lymph node aspiration and serology (DAT, ELISA, IFAT and western blot (WB)).

A cross-sectional survey was also conducted in Marbata village between 11 and 19 February 2002 and involved all village inhabitants. It included clinical examination and screening villagers with Leishmanin Skin Test, serology (DAT, ELISA, IFAT) and the Katex test.

Results Compared to microscopy, Katex had a sensitivity of 95.2%, with good agreement with microscopy but poor agreement with serological tests. It was also positive in the 2 confirmed visceral leishmaniasis patients co-infected with HIV infection. The test recorded a specificity of 94.4% in smear-negative patients subjected to test of cure, 89% in kala-azar patients during their follow-up, 100% in post-kala-azar dermal leishmaniasis (PKDL) patients who did not show clinical features suggestive of visceral leishmaniasis and in smear-negative confirmed malaria or tuberculosis patients. The test was also negative in all confirmed patients who had been actively followed-up 3 months after completion of their treatment.

While Katex had a specificity of 100% in healthy endemic and non-endemic controls, DAT was positive in 14% of the Katex-negative healthy endemic controls. Cross-reaction was not observed with confirmed tuberculosis or malaria patients.

Conclusion Katex is a sensitive, specific, rapid and simple addition to the diagnostics of visceral leishmaniasis, particularly at field level. Since the test seems to be specific for active disease, it can be particularly useful as a complementary test for the diagnosis of visceral leishmaniasis in smear-negative cases with positive DAT results and may also be helpful in the detection of treatment failure.

Background To date, there has been no reliable diagnostic test for active infections of visceral leishmaniasis. In fact, parasitological methods record high rates of false negative results, while serological tests based on the detection of anti-leishmanial antibodies are costly, need sophisticated techniques and are associated with cross-reactivity with other pathogens. The DAT is a sensitive, specific and simple test but its main disadvantage is its inability to distinguish...
between active disease, subclinical or past infections. A latex agglutination test for the detection of urinary antigen has recently been developed. The test has 100% specificity and a sensitivity of 81.4% in human urine samples, which is comparable to bone marrow aspiration. This study was conducted to evaluate the diagnostic validity of the test and its prognostic potential, particularly for remote areas with poor accessibility to health services.

Materials and methods
The study was conducted in three kala-azar treatment centres in Gedarif state, Eastern Sudan. The study population included new visceral leishmaniasis suspects, PKDL cases, patients investigated for a test of cure, and former kala-azar patients who reported for follow-up. Confirmed visceral leishmaniasis patients under treatment were subjected to follow-up for the evaluation of the therapeutic potential of Katex. Non-endemic and endemic healthy controls and cases of confirmed pulmonary tuberculosis and malaria were also included in the study.

Lymph gland aspiration was performed on visceral leishmaniasis suspects for the demonstration of amastigotes in stained smears, followed by culture on NNN and Evalus sloppy media. The strains were also characterized by isoenzymes electrophoresis. Blood samples were collected and tested by DAT, IFAT, ELISA and WB diagnostic tests. HIV screening was also performed. Urine samples were collected from all visceral leishmaniasis suspects, non-visceral leishmaniasis cases and healthy individuals in two sterile containers; one was used immediately for field testing and the other one was kept at -20 °C.

Katex test The test was performed by mixing 50 ml of the prepared latex reagent with 50 ml of the neat urine sample on a glass slide. The slide was rotated and rocked consistently for 2 minutes. Agglutination of ++ or more was considered positive.

Epidemiological study A house-to-house survey was conducted in Marbata village along the Atbara River. The survey included screening villagers for VL by clinical examination, collection of urine and blood samples, and interviewing them regarding sociodemographic characteristics, past history of visceral leishmaniasis, family history and other characteristics. They were also screened using LST and serological tests. The village inhabitants were stratified into those without evidence of infection (negative LST and serology), those with subclinical infection (negative LST and seropositive without past history of visceral leishmaniasis) and those with evidence of cured past infection (positive LST and seropositive). Lymph gland aspiration was performed for all patients with clinical features suggestive of visceral leishmaniasis.

Main study findings
The diagnosis of visceral leishmaniasis was confirmed with a positive lymph gland smear in 62 out of 180 new visceral leishmaniasis suspects (34.4%) and Katex recorded a sensitivity of 95.2%. There was good agreement between the two tests (Kappa coefficient = 0.65, P = 0.0), but poor agreement with serological tests (DAT, IFAT, ELISA, WB). Unlike DAT, no false-positive cases were recorded (100% specificity).

The follow-up of treated confirmed visceral leishmaniasis patients for 1-3 months and smear-negative ex-kala-azar cases showed conversion of Katex results to negative. Furthermore, evaluating its validity as a test of cure showed 100% sensitivity and 89.4% specificity. These findings are due to the rapid decline of the antigen level in the urine at week 12 resulting in conversion of the test before the end of the course of treatment. Other reports recommended the use of a competitive ELISA and rK39 for the prognostic evaluation of visceral leishmaniasis and the success of drug treatment. Obviously, due to its simplicity, the Katex may be more appropriate in this respect. Moreover, the test was also positive in the two parasitologically confirmed cases co-infected with HIV infection.

The field study conducted on 402 individuals screened by LST and serological tests confirmed the previous hospital-based study by showing good agreement between Katex and serological tests (Kappa coefficient = 0.65, P = 0.0), but poor agreement with serological tests (DAT, IFAT, ELISA, WB). Unlike DAT, no false-positive cases were recorded (100% specificity).

There was good agreement between the two tests (34.4%) and Katex recorded a sensitivity of 95.2%.

Although latent class analysis recommended treating clinical suspects in endemic areas based on positive DAT results, this study showed that LST was positive in 77.8% of smear-positive visceral leishmaniasis suspects with positive DAT results. Taking into consideration that positive LST rules out the diagnosis of active visceral leishmaniasis, accordingly, Katex could be a sensitive, specific, rapid test that is extremely useful to complement DAT for the diagnosis of unconfirmed visceral leishmaniasis cases.
Abstract

A household survey was conducted in two endemic villages and one non-endemic village for visceral leishmaniasis (VL) in South Syria whereby 483 children, aged 6 months to 6 years, were screened using the rK39 test. The aim of the study was to test the effectiveness of this test as an early diagnostic tool for VL, especially in remote areas with limited accessibility to health facilities. Only 10 positive cases were subjected to microscopic detection of amastigotes in bone marrow, while all positive cases were tested by ELISA. Screening of dogs was performed on a limited scale.

Results

Eighty children were positive for VL out of a total of 345 children in the two endemic villages, recording a prevalence of 23%. The prevalence was significantly higher in Assem village compared to Sour village (31.9% and 17%, respectively), while there was no significant difference between males and females regarding the prevalence of infection. All of the sera (N = 138) obtained from the control village, Ottaya, were negative (single band), suggesting high specificity. Sera from patients with acute VL were easily identified by rK39 testing, but patients with asymptomatic or self-healing infections had low or undetectable levels of anti-rK39 antibodies. Only 10 out of 80 positive children were symptomatic (12.5%). Symptoms included irregular fever (for one day), cough, diarrhoea, loss of weight and hepato-splenomegaly (2-3 cm). Only 22 out of the 80 positive cases by rK39 were shown to be positive by ELISA. This was thought to be due to the late seroconversion of ELISA, hence bringing evidence about early diagnosis of VL by rK39. When these positive cases were subjected to follow-up 9 months later using rK39 dipsticks, ELISA, and clinical examination they remained ELISA-negative, and none developed the full-blown disease.

Conclusion

Rapid diagnosis of VL can be performed using rK39 in symptomatic subjects since the test cannot differentiate between recent and old infection. However, the study could not provide evidence about rK39’s role in early diagnosis of VL.

Background

Serodiagnosis has been widely utilized for the diagnosis of visceral leishmaniasis (VL) in laboratories. However, there is a need for a simple, sensitive and specific diagnostic test for screening infected humans and reservoirs in the field, especially in remote areas with poor accessibility to health facilities. The rapid assay for the qualitative determination of antibodies to an antigen specific (K39) caused by members of the Leishmania donovani complex is considered a new, effective test. The use of this recombinant antigen (rK39) as a rapid tool for controlling VL is not currently performed in the countries of the Region.

The main objectives of this study were to test the efficacy of the rK39 test for early detection of VL cases among children under 5 years old in endemic villages, as well as testing its prognostic value in the follow-up of the subclinical and acute cases of VL in these villages.
Materials and methods

The study was conducted in 3 villages in Daraa, South Syria: Sour and Assem villages are endemic for VL, while the third one, Ottaya, was considered a control village. A household survey was conducted in the 3 study villages, whereby children aged between 6 months and 6 years were screened using the rK39 test. Children proved to be positive by the rK39 test were subjected to confirmed diagnosis by microscopic finding of amastigotes in bone marrow aspirates. This investigation was performed at Damascus hospitals because of the unavailability of necessary equipment in local health facilities. Serum samples (from positive children by rK39 dipsticks) were also tested by the ELISA method. Nine months later, positive cases were retested by rK39, ELISA and clinical examination. The aim of this follow-up phase was to study the prognostic potential of the test.

Technique of the rK39 test A drop of peripheral blood was placed on the dipstick. Flooding of the bottom protein with buffer allowed blood proteins to migrate upward. The positive reaction was obtained in 5-10 min, and it was indicated by the presence of an extra band "double bands" below the control band "single band".

ELISA method Breakable ELISA strips were sensitized with L. infantum soluble antigens (Bordier Kit). The wells were blocked with TBS-Tw. After removing the blocking solution, the wells were incubated with diluted serum samples, then washed 4 times with washing solution and incubated with conjugate. After removing the conjugate and washing, the wells were then incubated with the substrate. Finally, the strips were read in the personal-lab. ELISA reader.

Main study findings

Eighty children were positive for VL out of a total of 345 children in the two endemic villages, recording a prevalence of 23%. The prevalence was significantly higher in Assem village compared to Sour village (31.9% and 17%, respectively), and there was no significant difference between males and females regarding the prevalence of infection. On the other hand, all the sera (N = 138) obtained from the control village, Ottaya, were negative (single band).

The results of positive sera were further categorized into 3 groups: high, moderate, and weak. Sera from patients with acute VL were easily identified by the rK39 test but patients with asymptomatic or self-healing infections had low or undetectable levels of anti-rK39 antibodies.

Only 10 out of 80 positive children were symptomatic (12.5%), and among 3 out of these 10 cases, microscopic findings of amastigotes were positive. Symptoms included irregular fever, cough, diarrhoea, loss of weight and hepatosplenomegaly.

Microscopic finding of amastigotes in bone marrow aspirates was only performed in 10 out of the 80 positive cases due to a high refusal rate. All 10 tested cases proved to be positive for VL (sensitivity 100%).

There was poor agreement between rK39 and ELISA results. The number of positive sera by the ELISA technique was 22 out of the tested 80 sera (27.5%).

Screening of dogs was not adequately conducted due to the difficulty in catching wild dogs during visits to the volcanic, rocky hills. Only 9 sera from domestic dogs were collected during the field survey, and all were negative using the rK39 dipsticks. This was a study limitation.

Nine months following the initial survey, positive children were assessed clinically and serologically. All the children seemed to be healthy without evidence of full blown disease except 4 children with enlarged lymph glands.

Sera from children who were positive by the rK39 test were retested by ELISA. The number of positive sera by the ELISA test results were negative in all tested 55 cases, at 9 months of follow-up, which did not vary from earlier results. This latter finding indicate that ELISA is more specific in diagnosis of the disease since it recorded a lower frequency of false positive cases compared to rK39.

In conclusion, rK39 could be a rapid, sensitive and specific diagnostic test for visceral leishmaniasis in symptomatic cases living in remote areas with poor accessibility to health services. In disease endemic countries, VL could be easily misdiagnosed on clinical bases, therefore, screening these populations using rK39 test is recommended for early diagnosis and treatment of the disease thereby reducing its morbidity and mortality.
Objectives of this study were to study the epidemiological situation of kala-azar in Yemen, including the identification of the natural reservoir of the disease, particularly dogs. During a 2-year period, 94 cases of kala-azar with complete data were identified from the Sana’a area. The male-to-female ratio was 3:1 and the crude incidence rate was 13/100 000 over 2 years.

In a cross-sectional study of the population in 4 known endemic foci, over 285 individuals were randomly examined, and blood samples were taken and serologically tested for anti-leishmania antibodies by ELISA. Seropositivity rates ranged from 14% to 85.3%. There was a high risk of seropositivity with rural residence, ownership of dogs, open refuse dumps close to houses, stray dogs around the house and the presence of rodents in houses. Sixteen feral dogs were captured and amastigotes were observed in smears of livers and spleens of 4 of them. ELISA testing of their blood indicated that 75% of animals from the Benimansour area were positive, compared to 25% of those examined from Sharis area, giving a total prevalence of 50%.

Kala-azar is an important public health problem affecting mainly young children in northern parts of Yemen and infection is transmitted by the canine population. Strengthening control activities through case-finding, vector and reservoir control were emphasized.

Kala-azar has been reported from the northern part of Yemen since 1904, but nearly 4047 cases, mostly young children, were reported throughout the country during the 1980s. The causative organisms are *Leishmania donovani* complex and *L. infantum* complex, and the vectors are *Phlebotomus orientalis* and *P. arabicus*. This study was conducted aiming to determine the incidence of kala-azar in the study area, to determine the prevalence of seropositivity to Visceral Leishmaniasis (VL) among children in selected localities in Yemen, and to identify the natural reservoir of human (VL) in the areas, particularly dogs.

This study reported the failure of isolating leishmania organisms of confirmed kala azar cases on locally prepared NNN media. It was recommended that kala-azar should be made a notifiable disease in Yemen and that further studies should be organized to define more clearly the foci of human kala-azar, to trace and confirm possible reservoirs among canines and other wild animals as well as to study the sandfly vectors.
provinces) and from Sana'a city. Informed consent was taken, and all study participants completed a questionnaire that included sociodemographic data and potential risk factors for kala-azar.

**Serological assay** A 5 ml aliquot of venous blood was allowed to clot and the serum was separated and stored at -20 °C until serologic analysis was performed. All sera were tested for kala-azar using the ELISA technique. Samples with readings under 0.3 were considered negative, those with readings above 0.5 were positive, and readings in between these levels were considered equivocal (antibodies may be present but at nonsignificant levels). Subjects were divided into two groups based on their serology to study the risk factors for the disease.

**Collection and testing of canine specimens** Dogs were captured in two areas where cases of human visceral leishmaniasis had been identified (Benimansur and Sharis). Feral dogs were abundant in the study areas, living close to humans and wandering around farm buildings and houses, causing nuisance to the local population. A tented field laboratory was set up at each location for the purpose of providing a base where the animals could be examined. Sixteen dogs were captured, examined externally for signs of leishmaniasis, then dissected. Smears were prepared from any skin lesion detected, and from the liver and spleen of each dog. These were stained with Giemsa stain and examined microscopically for the presence of amastigotes. Biopsy specimens were collected aseptically from the livers and spleens, inoculated into a biphasic culture medium (NNN medium), then incubated at 21 °C for up to 6 weeks, and examined weekly for the presence of amastigotes.

Blood was collected from each dog and sera were separated and stored at -20 °C until assayed. Sera were tested for the detection of antibodies against (VL) by the ELISA test. Sera of dogs free from the disease were taken as reference, and 40% absorbance values of positive sera were considered positive.

**Main study findings**

During the 2-year period (1992-1993), a total of 94 cases were diagnosed in the study area. The male-to-female ratio was 3:1. The highest percentage of cases occurred in children under 5 years old (55%), and another 36% of cases occurred in the 5-9 year-old age group. The incidence of kala-azar in Sana'a was 13/100 000 over 2 years, with a significantly higher incidence in males compared to females; the highest monthly frequency was in March and the lowest from November to January. These figures far exceeded the reported number of cases in certain North African countries, where incidence rates of 0.34 and 0.24 per 100 000 have been reported from Algeria and Tunisia, respectively. The predilection of the disease to young children was in agreement with several reports and was attributed to impaired acquired immunity due to immature immune systems and inadequate nutrition. The higher incidence among males was attributed to increased exposures by males due to increased outdoor activity relative to females.

The seroepidemiological study revealed a seropositivity of 34.7% (99/285). The seropositivity was significantly higher in Sharis (85% of positive cases) compared to the other 3 regions that recorded comparable rates, ranging from 14-17.6% of positive cases. The high seropositivity rates were explained by the fact that most of individuals exposed to (VL) infection develop subclinical infections, while only a few of them will develop the clinical disease. There was a high risk of seropositivity with rural residence, ownership of dogs, open refuse dumps close to houses, stray dogs around the house and the presence of rodents in houses.

**Leishmaniasis infection in feral dogs** Lesions of possible leishmania origin were observed on the ears of five dogs in the Benimansur area, but no parasites were identified in the skin of these animals. Splenomegaly was observed in four and hepatomegaly in two dogs. Amastigotes were observed in Giemsa-stained smears of the livers and spleens of four dogs. ELISA testing of canine blood indicated that 75% from Benimansur were positive, compared to 25% from Sharis, with a total prevalence of 50%, which was higher than that reported from Saudi Arabia and Tunisia (19.3% and 6.03%, respectively). No growth occurred in the cultured liver and spleen of the dogs in NNN media, even for dogs found to be positive for amastigotes by liver and splenic aspiration. The prevalence of antibodies noted in the canine population may have been due either to infection with *L. infantum* complex and *L. donovani* complexes, whether past infection or active disease, or to antigenic cross-reaction with cutaneous leishmaniasis.
Abstract
The study aimed at the determination of the seroprevalence of visceral leishmaniasis (VL) in northern Morocco using the direct agglutination test (DAT). The study area consisted of 60 urban and rural clusters randomly selected from 8 provinces in northern Morocco. All children younger than 15 years of age were interviewed regarding sociodemographic variables, travel history, contact with dogs, and history of VL cases in the neighbourhood. Children were also subjected to clinical examination as well as to blood collection on filter paper, and blood samples were collected from 10% of the children.

Several sets of control sera were collected: from healthy subjects, confirmed VL cases, VL-positive cases with the immunofluorescent antigen (IFA) test, VL cases with negative IFA test sera from endemic areas, patients with positive anti-nuclear or anti-mitochondrial antibodies, and from patients with various infections. The DAT production was performed using the promastigotes of the Leishmania donovani infantum strain L dik 263. Filter-paper blood samples were extracted and plasma eluates samples were screened with DAT. The same strain of L. donovani infantum provided promastigotes for the immunofluorescent antigen IFA test.

Results
The seroprevalence of VL was 1.33%, with no statistically significant difference between rural (1.17%) and urban areas (1.51%). There was no significant association between the DAT positivity and gender, age, presence of domestic dogs at home or in the neighbourhood, and travel history. The DAT was proved to be a highly sensitive and specific test, stable at 4 °C when stored in the dark for up to 5 months. Providing training on the DAT production and the establishment of a laboratory for the continuous production and supply of the DAT to the different provinces were the main study recommendations.

Publications

Background
Central and southern Morocco are endemic for cutaneous leishmaniasis. Visceral leishmaniasis (VL) is found in the subhumid zones in the north of the country. Since 1991, VL cases have been increasing in the north province. A seroprevalence study was conducted by the research team using commercial reagents (Behring haemagglutination tests (HA)) in Tahla where most of cases have been reported. The study was conducted on 1203 filter-paper blood samples and reported a prevalence of 14%. These alarming results motivated the group to conduct the present study using the direct agglutination test (DAT) for the field diagnosis and determination of the seroprevalence of the disease in northern Morocco.

Materials and methods
Eight provinces were randomly selected from northern Morocco, and 60 clusters within these provinces were selected using systematic sampling in rural and urban areas. The clusters consisted of districts...
in urban areas, and localities in rural areas. All children younger than 15 years of age residing in the study area were surveyed by house-to-house survey. Data were collected according to a pre-designed questionnaire. The collected information included: sociodemographic variables, travel history, clinical examination, contact with dogs, and history of VL cases in the neighbourhood. Children were also subjected to blood collection on filter-paper (FPB), and blood samples on dry tubes were collected from 10% of the children. Blood samples were allowed to clot, and the serum was separated by centrifugation. Serum samples and air-dried FPB samples were stored at -20 °C until needed.

Control groups Several control sera were also collected: from healthy subjects, confirmed VL cases before and one month after treatment, VL-positive cases with the IFA test, VL cases with negative IFA test sera from endemic areas, patients with positive anti-nuclear or antimitochondrial antibodies, and from patients with various infections (cutaneous leishmaniasis, schistosomiasis, toxoplasmosis, malaria, hydatidosis, tuberculosis, leprosy, hepatitis, cytomegalovirus, HIV and syphilis).

DAT production The promastigotes of L. donovani infantum strain L dik 263 were grown at 26 °C in MEM medium containing Fetal Calf Serum (FCS), penicillin, and streptomycin. Mass production was done in MEM supplemented by FCS. The protocol of DAT production was according to the modified method described by El-Harith [1].

Filter-paper blood samples were extracted and plasma eluates samples were screened starting at 1/750 in V-shaped microtitre plates, 50 litres of antigen suspension were added, and the results visually read against a white background after 18 hours of incubation at room temperature. Serum dilution of 1/3000 or higher were suggestive of VL.

Immunofluorescent antigen The same strain of L. donovani infantum maintained in MEM media 10/FCS provided the promastigotes for IFA test. The promastigotes supension was collected, washed and fixed on IFA slides with absolute methanol for 5 minutes, then stored at -20 °C till used. The cut-off point for the control sera was 100.

Main study findings A total of 2101 filter-paper blood samples were collected, from which 1109 were from rural localities and 992 from urban districts. The IFA test was performed on 228 sera. All control sera were screened by the DAT and the IFA test. Confirmed VL cases were positive by both tests; out of 31 IFA-positive results, only 1 was positive with the DAT, mainly because of the lack of association with clinically proven VL; the IFA-negative sera were all negative with the DAT; out of 15 sera from patients with autoimmune disorders, only 1 was positive with the DAT; all sera from other infectious diseases were negative with the DAT. For the confirmed VL cases, DAT was proved to be a sensitive test (100%) with an acceptable specificity. The DAT was also proved to be stable at 4 °C when stored in the dark, since it was tested at different time intervals (1 up to 5 months).

The seroprevalence of VL was 1.33%, with no statistically significant difference between rural (1.17%) and urban areas (1.51%). Higher seroprevalence rates were reported from Tetouan (3.10%) and Kenitra (2.35%). It is worth mentioning that the IFA test proved to be an insensitive test as indicated by the high percentages of false-positive results. There was no significant association between the DAT positivity and gender, age, presence of domestic dogs at home or in the neighbourhood, and travel history.

Several recommendations were suggested to improve the quality of the DAT. Gelatin would be a good replacement for fetal calf serum in the DAT to reduce cost and improve its adaptation to field conditions. It was also suggested to consider the lyophilization and freezing of the antigen in order to increase the duration of storage and facilitate its transport to the provinces. Providing training on DAT production and the establishment of a laboratory for the continuous production and supply of the DAT to the different provinces were finally recommended.

References

Visceral Leishmaniasis

Sudan

Risk mapping of visceral leishmaniasis in Sudan

Gedaref State, Eastern Sudan

Study period: 2001–2002

Small Grants Scheme (SGS) 2000 No. 55

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Abstract
Visceral leishmaniasis (VL) is highly influenced by environmental factors. A study was carried out in 2001-2002 to map the distribution and risk of kala-azar in Sudan and investigate the history of infection by Leishmania donovani in Gedaref State, Eastern Sudan. Geographical Information Systems (GIS) were used to extract and map regression results for environmental variables of 190 villages in Gedaref State. VL incidence in each village was calculated from hospital records. Using logistic and linear multivariate regression analyses, models were developed to determine which environmental factors explain variability in VL presence and incidence. Average rainfall and altitude were proved to be the best predictors of VL incidence. The resulting models were mapped using GIS software predicting both VL presence/absence and incidence at any locality in Gedaref State. The models developed in this study provide detailed mapping of classified incidence of VL in Gedaref State. The fact that the models were derived from environmental variables resulted in the production of risk maps that could predict disease burden in areas not covered by the initial data. The risk maps produced from this study should be of great value for planning locations of treatment centres, for finding appropriate places for human settlement, and for deciding where to extend the control programmes. The produced models could be used to predict VL presence and incidence in other areas of Sudan where the disease is transmitted by the same vector. The novel approach of this study can be used for other parts of the world to predict and map VL transmitted by different vectors. Such studies would provide a global understanding of the VL problem and help prioritize control programmes.

Conclusions and implications of the study

- The models developed in this study provide detailed mapping of classified incidence of VL in Gedaref State. The fact that the models were derived from environmental variables resulted in the production of risk maps that could predict disease burden in areas not covered by the initial data.
- The risk maps produced from this study should be of great value for planning locations of treatment centres, for finding appropriate places for human settlement, and for deciding where to extend the control programmes.
- The produced models could be used to predict VL presence and incidence in other areas of Sudan where the disease is transmitted by the same vector.
- The novel approach of this study can be used for other parts of the world to predict and map VL transmitted by different vectors. Such studies would provide a global understanding of the VL problem and help prioritize control programmes.

Publications

Background
The present work describes an attempt to develop a detailed eco-epidemiology model for mapping the distribution and incidence of VL at the village level in Gedaref State in relation to different environmental factors. In addition to providing the first detailed map describing the occurrence and incidence of the disease in this important VL focus, the study also investigates how local variations in environmental factors affect the disease burden in different endemic villages.

Materials and methods
Study area
Gedaref State is bordered in the east by Ethiopia, in the south and west by the River Rahad, and in the north-east by the Atbara River. The region is a flat plain with almost no relief, and the principal soil type is vertisols. The climate is tropical continental, with an estimated annual rainfall of 400-1400 mm. The year is sharply divided between the rainy season, June-October, and the dry season, November-May. The mean minimum temperature is 21.0 °C in the rainy season and 18.3 °C in the dry season.
season; corresponding maxima are 37.3 °C and 40.6 °C. The natural vegetation is dry savanna woodland. The main indigenous trees are *Balanitis aegyptica*, *Acacia senegal*, *A. mellifera*, *Combretum spp.*, *Calotropis procera*, as well as some riverine vegetation. Along the river banks some fruit orchards are found. Dura, sesame, dockhon, and groundnuts are grown as cash crops over extensive areas. The human population of Gedaref State belongs to many ethnic groups, most of whom have a recent history of settlement in the region.

**Visceral leishmaniasis cases, human population and village location data** The case data analysed in this study were obtained from detailed records of 2 treatment centres established and operated by MSF-Holland in Umkra’a, situated close to the River Rahad, and in Kassab village, situated close to Gedaref town. Although a few patients were also diagnosed in Gedaref and Hawata hospitals and in other rural dispensaries, most patients have been referred to the 2 MSF VL treatment centres as a result of the high cost of treatment (estimated at US$ 170 per patient).

Data on cases and human populations were initially handled within Excel and SPSS software to calculate the numbers of cases of VL reported to the treatment centres during 1996-1999. Data were then entered in a new file containing the names of villages, their coordinates, councils, and the human population, and analysed to determine annual incidence (per 1000 people) in different villages. Coordinates of village locations were obtained from readings of a Magellan global positioning system and from maps produced by the South Kassala Agricultural Project.

**Environmental data** Environmental data corresponding to the coordinates of each of the study villages were extracted from a number of satellite sources and digital databases by Arcview GIS software with Spatial Analyst, and the public domain software WINDISP 3. The U.S. Geological Survey (USGS) hydrologic data set was used to obtain a detailed description of the topography of the area. Information on vegetation status was obtained from data archives of the vegetation sensor on board the French satellite system, SPOT. Ten daily images of 10 daily rainfall estimates for the years 1996-1998 were obtained from the Africa Data Dissemination Service and analyzed by Windisp 3 GIS software to obtain the average annual rainfall for each village. Soil types of different villages were read from a map produced by the South Kassala Agricultural Project and classified into 9 classes. Through the use of Arcview GIS software, the distance of each village from each of the 2 treatment centres and the two seasonal rivers was calculated.

**Statistical and GIS analysis of environmental and VL incidence data** Correlation analysis was performed to determine the relationship between the incidence of the disease and different environmental variables. Stepwise multivariate analysis was then carried out by binary logistic and linear regressions to determine predictor variables affecting the presence and incidence of VL, respectively. The produced models were then entered into the map calculator module of Spatial Analyst and used to create maps of probability of disease presence and incidence.

**Main study findings**

The mean incidence of VL per 1000 people was 6.91, with marked variation between different villages, ranging from 0 to 53.21. Clear clustering of high incidence villages and areas of low altitude and high rainfall zones was noticeable on the maps.

The results showed that distance from the river, the topography, rainfall, and the minimum Normalized Difference Vegetation Index (NDVI) are the main environmental variables independently associated with the distribution and incidence of VL in Gedaref State. These variables influence the populations of the vector and reservoir hosts of *L. donovani* by affecting other microclimatic factors in the area. *Phlebotomus orientalis* is known to thrive in habitats characterized by the presence of *B. aegyptica* trees, *A. senegal* trees, and vertisols. The vector was also found to inhabit a "climate space" of rainfall of 400-1200 mm and of annual mean maximum daily temperatures of 34-38 °C. Because most of the region is covered by vertisol, it is not surprising that this factor did not seem to affect the distribution of disease within the region.

In all the analysis carried out in this study, annual rainfall appeared to be the most important predictive variable affecting both the probability of presence and incidence of the disease. Rainfall may affect the vector and reservoir hosts by affecting the vegetation, the temperature, and the relative humidity.

**Conclusions and recommendations**

The models developed in this study provide detailed mapping of classified incidence of VL in Gedaref State. The fact that the 2 models were derived from environmental variables resulted in the production of risk maps that could predict disease burden in areas not covered by the initial data. It is suggested that the novel approach of this study can be used for other parts of the world to predict and map VL.
Abstract

There have been contradictory reports regarding the types of visceral leishmaniasis strains prevalent in Iraq; and this variability might explain the observed differences in the severity of the disease and its response to chemotherapy. The present study was conducted to identify the new isolated visceral stocks of leishmania, and its relation to the visceral stocks from Ethiopia and the Mediterranean region. Seven human visceral stocks isolated during the period 1988-1990 and obtained from the cryobank were subjected to isoenzymes characterization. During the period 1993-1994, primary isolation of leishmania was attempted from bone marrow aspirates taken from 15 children diagnosed clinically and/or by indirect immunofluorescent antibody test (IFAT). Primary isolation was attempted again during the period November 1994-May 1995. Bone marrow cultures were done for 33 patients (4-28 months) whom were proven to have kala-azar by IFAT and/or bone marrow smear. Several manipulations were done trying to isolate the parasites.

Results

According to the zymograms obtained, the 7 isolates were divided into 3 groups (African, Mediterranean and mixed). These results confirmed the coexistence of the Mediterranean *Leishmania donovani infantum* and the African *L. donovani* in Iraq. In comparison with earlier results, there was a change in parasite type, which may account for the recent difficulty in parasite isolation.

Conclusions and implications of the study

- Isoenzyme characterization confirmed the coexistence of the African *L. donovani* together with the Mediterranean *L. donovani infantum* in Iraq.
- Bone marrow aspirates of children diagnosed clinically and/or by indirect immunofluorescent antibody test (IFAT) showed a high frequency of positive primary cultures. However, there was difficulty in the primary isolation, which was explained by strain differences.
- Bone marrow cultures done for children proven to have kala-azar by IFAT and/or bone marrow smear yielded negative results. In comparison with earlier results, there was a change in parasite type, which may account for the recent difficulty in parasite isolation.

Background

The clinical picture of kala-azar in Iraq is of the infantile type, which is primarily seen in the Mediterranean area. It has been noticed by many investigators that children with visceral leishmaniasis show differences in the severity of the disease as well as in their response to chemotherapy. An earlier study conducted in Iraq, using isoenzyme electrophoresis, reported that only 8% of the human visceral stocks were identical to a WHO reference stock (MHOM/ET/67/HU3) from Ethiopia, while 92% of the stocks revealed a new GPI enzyme variant [1]. Other studies reported the heterogeneity of visceral stocks in the country and their dissimilarity from Sudanese, Iranian, and Tunisian stocks, and their similarity to reference stocks from Ethiopia [2]. In view of the controversy between different reports, there was a need to conduct the present study in order to identify the new isolated visceral stocks of leishmania, and its relation to the visceral stocks from Ethiopia and the Mediterranean region (MHOM/ET/67/HU3 and MHOM/TN/80/IPT1, respectively).
Materials and methods

Seven human visceral stocks isolated during the period 1988-1990 and obtained from the cryobank of the Medical Research Centre at Saddam College of Medicine were subjected to gel electrophoresis of 5 enzymes: PGM, SPGDH, MDH, GPI and ME, together with the reference stocks from Ethiopia and Tunisia: MHOM/ET/67/HU3 and MHOM/TN/80/IPT1.

During the period 1993-1994, primary isolation of leishmania was attempted from the bone marrow aspirates of 15 children diagnosed clinically and/or by indirect immunofluorescent antibody test (IFAT). A few drops of the aspirate were usually introduced into a semisolid medium or biphasic medium (brain heart infusion agar containing 0.8% glucose and 10% defibrinated rabbit blood as the solid phase with Locke’s overlay). The cultures were incubated at 26 °C and examined every 3 days. If no parasites were observed during the 3-week incubation period, they were discarded. The first subcultures were not as good as the primary cultures, and on the third subcultures the promastigotes disappeared. In an attempt to rescue the primary cultures, 2 stocks were inoculated intraperitoneally in mice. After 1 month the mice were dissected and the parasites were recovered in cultures of the liver and spleen after 5 days. No evidence of growth was noted in the third subculture.

Primary isolation was attempted again during the period November 1994-May 1995. Bone marrow cultures were done for 33 cases (4-28 months) proven to have kala-azar by IFAT and/or bone marrow smear. The following manipulations were done to try to isolate the parasites:

1. A semisolid medium containing 20% defibrinated rabbit blood was used.
2. Eight aspirates were introduced into the medium as well as on the same medium but containing 20% hamster liver or spleen extract. The extract was prepared by adding Locke’s solution to the tissue (1/5, v/v) followed by homogenization and centrifugation.
3. Eight bone marrow aspirates were introduced into EDTA-containing tubes to prevent blood coagulation. The samples were centrifuged and washed twice with PBS. The pellets were re-suspended in Locke’s and inoculated into the semisolid medium as well as introduced intraperitoneally into mice (2 per sample).
4. Four bone marrow aspirates were diluted with PBS containing EDTA as anticoagulant, centrifuged, washed and resuspended in PBS, then injected intraperitoneally into mice.
5. Glass syringes were used in 6 cases to exclude the possibility of monocyte sticking to the plastic surface of the syringe.

Main study findings

Isolates obtained during 1988-1990

According to the zymograms obtained, the 7 isolates were divided into 3 groups:

- Group 1 included the stocks RTC7, RTC8, RRL45 and the reference stock IPT1.
- Group 2 included only one stock (RRLL7) which was identical to the reference stock HU3.
- Group 3 included the stocks RRL34, RRL36 and RRL37. These stocks had zymograms identical to that for IPT1, except for GPI which was identical to HU3.

These results confirm the coexistence of the Mediterranean L. donovani infantum and the African L. donovani. The former was undetected in parasite isolations made during the period 1978-1984. This change in parasite type may account for the recent difficulty in parasite isolation. Group 3 may represent hybridized strains evolved as a result of the coexistence of the parent parasites in the vector or the host.

Recent results

During the period 1993-1994, 12 out of 15 bone marrow aspirates (80%) showed positive primary cultures. There was difficulty in the primary isolation, which was explained by strain differences.

Primary isolation was attempted again during the period November 1994-May 1995 and the results showed the presence of few parasites in the primary cultures of 3 out of the 33 aspirates incubated in the semi-solid medium. None of the manipulations improved parasite recovery. Aspirates from 30 cases given intraperitoneally into mice revealed parasites in liver smears when the animals were sacrificed 3-6 weeks later, but the cultures were negative. No stock was thus obtained from these 33 cases of kala-azar.

These results indicate a change in parasites towards strains difficult to isolate. It has to be mentioned that isolation of the parasite from the sandfly vector could not be performed because of difficulties in conducting field visits created by the sanctions.

References


Abstract

The present study aimed at identifying the possible role of humans as a reservoir of leishmaniasis and its implication in the epidemiology and control of the disease.

Study area: Three groups of populations living in different areas were selected; the first group consisted of the entire population of a village in Karshola Town, Nuba Mountains area, western Sudan; the second group consisted of 89 high-risk individuals from a highly endemic area in eastern Sudan, called Dinder Park; the third comparison group was from Salala Village, eastern Sudan.

Data were collected from the 3 studied groups by leishmanin skin test, serology and clinical examination of the population. The initial visit was followed by 2 subsequent visits, before and after the rainy season, and follow-up data were collected. The presence of parasite DNA was detected by PCR and subsequent southern blotting. The kDNA was amplified using species specific primers.

Results

The leishmanin skin test was positive in 50%, 53% and 70.5% of the populations of Karshola, Salala and Dinder, respectively. Serology was positive in 0.3%, 9.6% and 44.4%, respectively. PCR was positive in 28.6% of the Dinder Park population only and there was a significantly higher proportion of clinical kala-azar cases in Dinder Park (15.8%) as compared to Karshola and Salala (0.13% and 3.85%, respectively). This study allowed the identification of new foci of visceral leishmaniasis in the Nuba Mountains, western Sudan, where low transmission is taking place. Among the high-risk groups living in endemic areas, the geographical origin and ethnicity of the subjects were superadded factors.

Conclusion

These results confirm the general preponderance of subclinical infection to clinical disease, but pointed as well to the susceptibility of certain ethnic and geographical populations to kala-azar.

Publications


Training

Dr Nazar M Abdalla received a WHO/TDR research fellowship in Dr Douglas Barker's laboratory in Cambridge. He learned several techniques such as labelling of probes and hybridization that he introduced in 2 institutions in Sudan: the Unit of Genetics and Molecular Parasitology, National Health Laboratory, Khartoum, and the University of Gezira, his home institution.

Background

Leishmaniasis is one of the major health problems in the Sudan. Several studies have been conducted, mainly in the eastern and southern Sudan. In western Sudan, foci of leishmaniasis were recognized during the 1970s. Studies performed in the different endemic areas have reported that the majority of the inhabitants of these areas contract infection but do not develop the clinical disease, i.e. remain subclinical. However, there was evidence that a considerable proportion of these individuals harbour the leishmania parasite in their peripheral blood [1]. This finding was the basis of conducting the present study that aimed at identifying new foci of visceral leishmaniasis in the Nuba Mountains, western Sudan.
the possible role of humans as a reservoir of leishmaniasis and its implication in the epidemiology and control of the disease.

Materials and methods

Three population groups living in different areas were selected: the first group consisted of the entire population of a village in western Sudan, the second group consisted of high-risk individuals living in a highly endemic area in eastern Sudan, and the third group was from Salala Village in eastern Sudan.

The first study site was selected from the health records of the Nuba Mountains area. Accordingly, an accessible village with the highest prevalence of leishmaniasis with a population of 1000 individuals was selected. The village, El Mougaful near Karshola Town, western Sudan, was visited in order to take the consent of the population, perform the census, and collect baseline information in a pre-designed questionnaire. Leishmanin skin test, serology and clinical examination of the population were also carried out. The initial visit was followed by 2 subsequent visits, before and after the rainy season, and follow-up data were collected.

Another group consisting of 89 high-risk individuals were from a highly endemic area in eastern Sudan. These individuals were mainly healthy game wardens and army soldiers, and they were subjected to PCR, leishmanin skin test and serology to determine the incidence of kala-azar and sub-clinical infection among them. The presence of parasite DNA was detected by PCR and subsequent southern blotting. The kDNA was amplified using species-specific primers developed by the group of Dr DC Barker in Cambridge. PCR products of 800 bp were obtained in positive samples. Comparison was also made with data obtained from Salala Village in eastern Sudan where a 6-year longitudinal study on kala-azar was just completed during the conduction of the present study.

Main study findings

The incidence of the clinical disease (kala-azar) in El-Mougaful village, Karshola, was around 0.1%, and the leishmanin rate was high (50%), although there was no evidence of cutaneous leishmaniasis. The direct agglutination test (DAT) was positive in less than 0.3% of the villagers and positive cases recorded a very high titre (>25 000). PCR was performed for the entire village. And despite the suboptimal quality of the DNA extracted, positive samples of Leishmania donovani were obtained and confirmed by southern blotting technique.

Preliminary PCR screening in Khartoum was successful in some samples, indicating the presence of the leishmania parasite. There were difficulties in analysing the larger sample in Cambridge mainly due to the low parasitaemia of the area and inadequate sampling methods.

In Dinder Park and Karshola Village, 2 sets of serological tests were used, DAT and k39. The latter was more sensitive as evidenced by its picking up subtle cases of infection with Leishmania donovani.

This study allowed the identification of new foci of visceral leishmaniasis in the Nuba Mountains in western Sudan, where low transmission is taking place. The 3 different comparison foci were situated within the same Acacia belt running through central, eastern, and western Sudan. The Leishmania-positive rates were comparable in the 3 studied areas. It appears that the onset of outbreaks depends on a critical mass of susceptible individuals and the availability of human carriers (reservoir). Among game wardens of Dinder Park, the geographical origin and ethnicity of the subjects were additional factors. These results pointed to the susceptibility of certain groups to the disease. It was recommended to confirm this observation by future in-depth studies intensively investigating the role of the human host in the epidemiology of the disease.

References

Abstract
The objectives of this study were to characterize parasite isolates from visceral leishmaniasis (VL), post kala-azar dermal leishmaniasis (PKDL) patients and paired isolates from patients who developed VL followed by PKDL, as well as the parasite determinants of PKDL.

A total of 270 confirmed VL patients, aged 3-12 years old, and residing villages in Gedaref State, Eastern Sudan, were included in the study. Lymph node aspirates were cultured on NNN media.

Two methods of DNA extraction were used: direct lysis of promastigotes and the Chelex extraction method. The DNA band profiles and restriction fragment length polymorphism were determined using PCR. Electrophoresis was done on promastigotes, growing VL and PKDL were lysed and the lysate separated, then the protein bands were stained. ELISA was used for the measurement of IgM, IgG and IgG subclasses in both VL and PKDL sera samples.

Results
The culture of the parasites on the NNN medium revealed that 44.8% of parasites isolated from VL grew on this medium compared to 26.7% of those isolated from PKDL patients. A total of 270 confirmed VL patients, aged 3-12 years old, and residing villages in Gedaref State, Eastern Sudan, were included in the study. Lymph node aspirates were cultured on NNN media.

Two methods of DNA extraction were used: direct lysis of promastigotes and the Chelex extraction method. The DNA band profiles and restriction fragment length polymorphism were determined using PCR. Electrophoresis was done on promastigotes, growing VL and PKDL were lysed and the lysate separated, then the protein bands were stained. ELISA was used for the measurement of IgM, IgG and IgG subclasses in both VL and PKDL sera samples.

Conclusions and implications of the study
- Culture of the parasites on NNN medium was successful in 44.8% of parasites isolated from VL compared to 26.7% in PKDL patients.
- The Chelex method was proved to be superior to the direct lysis method in DNA extraction and amplification.
- All isolates typed by PCR were identified as *Leishmania donovani* complex with amplification of the characteristic 800 bp band as compared with the Ld 2S reference strain.
- The PKDL restriction profiles were heterogenous, indicating that PKDL is caused by more than one *Leishmania donovani* strain (clone). This finding has not been previously reported and has an important epidemiological implication.
- Two protein bands were invariably detected in VL and PKDL isolates, but the 65 000 dalton band was more intense in PKDL isolates.
- Anti-leishmania IgM titres were significantly higher in VL patients compared to normal controls and there was no significant difference between VL and PKDL regarding IgG levels, but they were significantly higher in these patients compared to resistant VL patients. IgG1, and IgG3 subclasses were significantly higher in all VL and PKDL compared to IgG2 and IgG4.

Background
Post kala-azar dermal leishmaniasis (PKDL) is a serious complication of visceral leishmaniasis (VL), and is characterized by skin lesions (papules, macules, and/or nodules) on the exposed parts of the body. Cases were also reported in individuals without a history of clinical VL. PKDL is considered of epidemiological importance as cases provide a rich source of infection for sandflies. Furthermore, it is often clinically misdiagnosed as leprosy, thereby delaying proper management of the condition.
The pathogenesis of PKDL is not known and several hypotheses have been proposed including a unique parasite species or strain, differential expression of peculiar genes in the parasite during the pathogenesis of PKDL, and the involvement of host immune response. The existence of a peculiar parasite was ruled out. Parasites isolated from PKDL patients had an identical isoenzyme pattern of VL isolates, indicating that PKDL is caused by a typical L. donovani parasite. However, the cloning of a DNA minicircle sequence of 560 bp from a Leishmania donovani PKDL isolate was proved to be specific for PKDL. Regarding the role of the host immune response, immunological investigations of PKDL lesions showed marginal to massive infiltration of mononuclear cells depending on the duration of illness and lesion type. Analysis of the humoral immune response in VL and PKDL patients showed the induction of leishmania specific antibodies of IgG class with the domination of IgG1, followed by IgG2, IgG3 and IgG4. The subclasses have different specificity to variable leishmania antigens. Furthermore, Interleukin IL 10 has been identified as an immunological predictor of PKDL.

The objectives of this study were to characterize parasite isolates from VL, PKDL patients and paired isolates from patients who developed VL followed by PKDL, as well as the parasite determinants of PKDL.

**Materials and methods**

During 12 field visits, 270 individuals out of 342 suspected VL patients had a confirmed VL diagnosis using the direct agglutination test (DAT). The majority of patients (90%) were aged 3-12 years, and they were residing in villages in Gedaref State, eastern Sudan. All patients were treated with Pentostam under medical supervision.

**Parasite cultures** Lymph node aspirates from the 270 confirmed VL patients and 30 PKDL patients were cultured on NNN media as a primary isolation medium. Mass cultures were done using RPMI 1640 media supplemented with 20% heat-inactivated fetal calf serum (FCS) and antibiotics. Growth of the parasite was detected by examining the culture under an inverted microscope.

**Characterization of the isolates** Two methods of DNA extraction were used: direct lysis of promastigotes and the Chelex extraction method.

**Polymerase chain reaction** Two sets of primer were used: AJS3/BDY and RH1/RH2. This was followed by PCR amplification, then the amplified DNA fragments were separated, stained and visualized to determine the DNA band profiles.

**Restriction fragment length polymorphism (RFLP)** A minicircle of selected L. donovani isolates was amplified by PCR using AJS3/DBY primers. The product was digested with a restriction enzyme and the restriction profile examined.

**SDS PAGE** Sodium dodecyl sulfate polyacrylamide gel electrophoresis was done using the Lamellae method. Whole promastigotes of growing VL and PKDL were lysed and the lysate separated, then the protein bands were stained.

**Analysis of antibody profiles of visceral and PKDL patients** ELISA was used for the measurement of IgM, IgG and IgG subclasses in both VL and PKDL sera samples.

**Main study findings**

Culture of the parasites on NNN medium revealed that 44.8% (121/270) of parasites isolated from VL grew on this medium compared to 26.7% (8/30) of those isolated from PKDL patients, and two isolates grew directly from skin snips of PKDL lesions. The Chelex method was proved to be superior to the direct lysis method in DNA extraction from 121 VL and 8 PKDL isolates and their amplification. Moreover, the amplified bands obtained by this method were intense and sharp. All isolates typed by PCR were identified as L. donovani complex with amplification of the characteristic 800 bp band as compared with the Ld 2S reference strain.

The PKDL restriction profiles were heterogenous, indicating that PKDL is caused by more than one Leishmania donovani strain (clone). This finding has not been previously reported and has an important epidemiological implication.

More than 5 protein bands were identified in all the isolates; out of these bands 2 were invariably detected in VL and PKDL isolates. The 65 000 dalton band was more intense in PKDL compared to the VL isolates.

Regarding the antibody profile of patients, anti-leishmania IgM titres were significantly higher in VL patients compared to normal controls. Anti-leishmania IgG antibodies were significantly higher in VL and PKDL samples compared to resistant VL patients; on the other hand, there was no significant difference between VL and PKDL regarding IgG levels.

Study of the IgG subclasses revealed the following: IgG1 was significantly higher in all VL and PKDL patients and in 90% of resistant VL (RVL) patients; IgG2 in all PKDL patients, 91% of VL and 70% of RVL patients; IgG3 in all VL, 93% PKDL patients and in 70% of RVL patients; and IgG4 in 89% of VL and 50% of RVL patients.
Visceral Leishmaniasis Vaccine trial

Islamic Republic of Iran

Field efficacy vaccine trial with alum-precipitated Leishmania major antigen against canine visceral leishmaniasis in Meshkin Shahr district, north-west Iran

Islamic Republic of Iran
Meshkin-Shahr district, North-west Iran

Study period: January 1999–July 2001

Small Grants Scheme (SGS) 1998 No. 38

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Abstract
In this study, 406 dogs with owners and with no anti-Leishmania antibodies from Parikhan village in Meshkin-Shahr district were physically examined. Three hundred and fifteen healthy seronegative dogs with no response to leishmanin were selected and randomly injected by either alum-precipitated autoclaved Leishmania major (ALM) vaccine (200 µg) of Leishmania protein precipitated in roughly 620 µg of aluminum hydroxide mixed with BCG (roughly each dog received 2 million CFUs) or injected with PBS as a control. Blood samples were taken from the dogs 4 months post injection and were skin tested with leishmanin (LST). The injected dogs were followed-up for 16 months.

Results
The examination of the site of injection showed that the vaccine is well tolerated and no severe reaction was observed. The LST conversion rate was significantly higher in the vaccinated group compared to the placebo group. The efficacy evaluation performed at 16-month post vaccination indicated that administration of this vaccine plus BCG had 54.6% protective fraction effects against canine visceral leishmaniasis.

Background
Human visceral leishmaniasis (VL) caused by Leishmania infantum is endemic throughout the north-western and southern parts of Iran. Dogs are the main reservoir of infection and many seropositive dogs exist in endemic areas. However, treatment of infected dogs with antimonials proved to be noneffective. In view of the growing public health importance of zoonotic visceral leishmaniasis (ZVL) and problems encountered in its control, new preventive strategies seemed necessary. Several studies performed on dogs experimentally and naturally infected with L. chagasi and L. infantum indicated that many animals survive the infection and develop a cellular immune response that probably resulted in resistance. These findings suggested that a vaccine against canine visceral leishmaniasis is quite feasible. This study was conducted to test the efficacy of a single injection of aluminium hydroxide-autoclaved Leishmania major (Alum-ALM) vaccine mixed with BCG against canine visceral leishmaniasis, in Meshkin-Shahr, Iran.

Materials and methods

Study site
Parikhan village is located 5 km west of Meshkin-Shahr, Ardebil province, north-western Iran. The village has a population of 8000, and has more than 800 leashed dogs. The selection of the study site was based upon previous reports on the seropositivity of its children and dogs reservoir to infection.

Vaccine
The alum-precipitated Leishmania major vaccine (Alum-ALM) was produced at Razi Institute, Hesarak, Iran. Autoclaved Leishmania major vaccine (ALM) was produced from promastigotes of L. major (MRHO/IR/76/ER) from a seed bank. The promastigotes were grown in RPMI supplemented with 20% FCS at 25 °C in a roux bottle. The promastigotes were harvested at the stationary phase, and were washed 5 times with phosphate-buffered saline (PBS) and stored at -70 °C.

Aluminium hydroxide containing 22.22 mg aluminium ion per millilitre was produced and autoclaved at 121 °C for

Conclusions and implications of the study

This study reported the preliminary results in the development of a vaccine for canine visceral leishmaniasis. The leishmanin skin conversion rate among vaccinated dogs was higher than for the placebo group.

The vaccine was well tolerated and no severe reaction was observed.

Administration of this vaccine to canids provided a protective effect of 54.6% against canine visceral leishmaniasis.

Further evaluation of this vaccine trial is recommended.
15 minutes. The alum solution was mixed with an equal volume of ALM and aliquoted 0.9 ml in each vial of small vials of 9 ml capacity. The vials were autoclaved under similar conditions, the final concentration of Leishmania protein is 3.6 mg/ml. Freshly re-suspended BCG was added and mixed well just prior to injection, and each dog received 200 µg of Leishmania protein precipitated in roughly 620 µg of aluminium hydroxide.

BCG and the leishmanin skin test (LST) were produced at the Pasteur Institute, Tehran, Iran. Leishmanin was prepared from the same isolate of L. major that was used for the vaccine preparation.

**Experimental design** This study was a double-blind randomized trial. Eligibility criteria for the dogs were: age older than 1 month, ownership of the dog during the study period (at least 2 years); freedom from current disease based on veterinary examination, and negative antibody titre against L. infantum.

A total of 406 dogs were physically checked by a vet, and blood samples were collected from 360 dogs. LST indicated that 325 dogs had no antibody titre against L. infantum antigens. Based on physical examination and serological results, a total of 320 dogs were randomly allocated to either of 2 vaccines: 0.1 ml of Alum-ALM vaccine (200 µg protein per dose) mixed with BCG (2x106 colony forming units (CFU) per dose) or with 0.1 ml of PBS alone. A single injection was administered intradermally in the forearm of the dogs. A safety follow-up was performed 30-45 days post vaccine injection. The injected dogs were checked by a vet and the site of inoculation was examined. The type and size of the lesion induced by the injection was recorded. Blood samples were collected from 242 dogs 4 and 16 months post vaccination to determine the humoral immune response induced by the vaccine.

The control group were dogs that were vaccinated and did not develop the disease and non-vaccinated dogs (with or without disease).

**Main study findings**

**Post-vaccination follow-up** A total of 246 injected dogs were checked after vaccination, and in more than half of injected dogs, no lesion was detected at the injection site. However, an ulcer or an induration were induced by the injection in 45.3% and 2.9% of dogs, respectively. The size of the lesion (ulcer or induration) ranged from 2-10 mm in diameter.

**Immunological studies** The antibody response measured by ELISA post-vaccination revealed that 95.4% of dogs were negative compared to 95.9% when tested by the DAT. Leishmanin skin testing post vaccination assessed the cell mediated immunity response induced by vaccination. The LST revealed that in 246 tested dogs, 191 were negative (77.6%), while the remainder showed a response ranging from 5-10 mm in diameter. Blood samples collected from every injected dog 16 months post vaccination were used to detect anti-Leishmaniais antibodies by DAT and ELISA techniques. The results showed that 5.9% of dogs injected with ALM mixed with BCG and 13% of dogs receiving PBS alone, were seroconverted.

**Conclusions and recommendations**

Alum ALM mixed with BCG had 54.6% protective fraction against canine VL. Furthermore, the vaccine prepared proved to be a safe vaccine. These results provide an initial step in the vaccine preparation for dogs.
Abstract
Field collected sandflies were tested for susceptibility to different insecticides using WHO test kits. The susceptibility of Phlebotomus papatasi adults to different insecticides was tested in 2 successive years, the summers of 1994 and 1995. Tests were carried out under field conditions. Insecticides tested were DDT, dieldrin, malathion, propoxur, permethrin and deltamethrin, representing different insecticide groups.

Results
The sandflies which were tested not only showed the highest susceptibility to insecticides among all other Egyptian sandflies, but also among sandfly populations tested in several other parts of the world. The high susceptibility level of the adult P. papatasi population in this study was attributed to the fact that this area had no history of insecticide applications. The fact that the adult P. papatasi population tested in this study was still highly susceptible to DDT and dieldrin raised the possibility of using these 2 compounds in sandfly control programmes.

Conclusions and implications of the study
Sandfly collection revealed that Phlebotomus papatasi was the only sandfly species identified in the study area.

The Phlebotomus papatasi population was highly susceptible to all insecticides: DDT (chlorinated hydrocarbons), dieldrin (cyclodiene), malathion (5%) (organophosphate), propoxur (0.1%) (carbamate), deltamethrin (0.025%) and permethrin (0.25%) (pyrethroids).

The high susceptibility level of the adult Phlebotomus papatasi population in this study could be attributed to the fact that this area had no history of insecticide applications.

The fact that the adult Phlebotomus papatasi population tested in this study was still highly susceptible to DDT and dieldrin raised the possibility of using these 2 compounds in sandfly control programmes.

Background
In Egypt, phlebotomine sandflies are important vectors of the phlebotomus fever (sandfly fever) virus and 2 forms of Leishmania, Leishmania major and L. infantum [1]. L. major is transmitted by the sandfly Phlebotomus papatasi, the vector of cutaneous leishmaniasis (CL) in North Sinai, and L. infantum is transmitted by P. langeroni, the vector of visceral leishmaniasis (VL) in Alexandria. An outbreak of a CL epidemic in North Sinai was documented in 1991 [1]. Application of residual insecticides remains the principal method for the control of sandflies, especially in developing countries. Since sandflies have not been principally targeted in control operations, the control of sandflies has been often a by-product of antimalarial spraying. In addition to DDT, malathion, fenitrothion, propoxur, dieldrin, lindane and synthetic pyrethroids have been successfully used for the control of sandflies. However, the spectrum of susceptibility of sandflies to a range of insecticides has not yet been studied. The objective of this work was to determine and monitor the susceptibility level of sandfly field populations in the Sinai peninsula to different insecticides.

Materials and methods
Adult sandflies were collected from Sad El-Rawafaa, North Sinai. CDC light traps were used for overnight insect collection, and insects were allowed to settle for a few hours for conditioning together with 10% sucrose solution. Only active healthy insects were tested against a series of insecticide concentrations and different exposure times. Tested insects were identified as Phlebotomus papatasi...
Insecticides Impregnated papers with different concentrations of DDT (chlorinated hydrocarbons), dieldrin (cyclodiene), malathion (5%) (organophosphate), propoxur (0.1%) (carbamate), deltamethrin (0.025%) and permethrin (0.25%) (pyrethroids) were supplied by the WHO. For control tests, insects were exposed to impregnated papers prepared in the laboratory: resiella oil (for organochlorines and pyrethroids) and olive oil (for organophosphates and carbamates).

Exposure techniques WHO standard test techniques were used [3]. The sandflies were subjected to different concentrations, or different exposure times at the same concentration. At least 3 replicates of 20 to 25 insects were tested for each concentration or exposure time. Mortality counts were taken after a 24-hour recovery period. Control insects were exposed at the maximum exposure time used for the tested insects.

Statistical analysis Test mortality percentages were corrected using Abbott’s formula (control mortality percentages >5%) and subsequently analysed by probit analysis. Goodness of fit of regression lines was measured by the Chi-square test. LC\textsubscript{50} or LT\textsubscript{50} values were estimated and the slope values of the regression lines were calculated.

Experimental conditions Testing was performed during the summer (May-October) in the same locality for 2 consecutive days, in 1994 and 1995. Seasonal peaks of sandfly abundance in this area were reported to occur between June and September. However, in the 1994 season, the adult density was unexpectedly low due to floods in the study area so that tests with DDT and dieldrin could not be performed. Also, the number of insects used for every test was relatively smaller than that used in the 1995 season. Susceptibility tests were carried out in a room which was specially prepared and free of insecticide. During the test, the temperature was maintained at 27-30 °C and the relative humidity (RH) at 80-90%.

Main study findings
The results obtained in this study revealed that the tested population was highly susceptible to all insecticides as evidenced by the discriminating concentrations or times. They also indicated that the susceptibility of adult sandflies to different insecticides did not show any significant difference between 1994 and 1995. However, little difference (less than 3-fold) in the LT\textsubscript{50} values was detected with malathion and propoxur between the 1994 and 1995 tests. On the other hand, there was no remarkable difference between the LT\textsubscript{95} values of the same insecticides. The high susceptibility level of the adult *P. papatasi* population in this study could be attributed to the fact that this area had no history of insecticide applications, unlike other Egyptian regions that were subjected to mosquito control measures such as the El-Agamy area, or to insecticide applications for the control of agricultural pests as in the Nile Delta and Giza Governorate. The sandflies which were tested not only showed the highest susceptibility to insecticides among all other Egyptian sandflies, but also among sandfly populations tested in several other parts of the world. The fact that the adult *P. papatasi* population tested in this study was still highly susceptible to DDT and dieldrin raised the possibility of using these 2 compounds in sandfly control programmes. The slight differences in susceptibility based on LC\textsubscript{50} and LT\textsubscript{50} reported in this study do not reflect a real resistance difference because there were no significant differences among LC\textsubscript{95} or LT\textsubscript{95} values. This slight difference may have been due to fluctuations in tolerance and seasonal variations in the population.

References
Abstract

Previous studies have confirmed the efficacy of Scalibor dog collars in protecting against sandfly bites. This justified the same research team to carry out a community-based intervention trial using deltamethrin-impregnated dog collars during the transmission season in the endemic zone of zoonotic visceral leishmaniasis. An intervention study was conducted in Kalaybar and Meshkin-Shahr, north-west Iran, whereby children aged 1-10 years were surveyed using the DAT and leishmanin skin test (LST). All domestic dogs were also screened by the DAT. Villages were matched for disease prevalence and villages in each pair were randomly allocated as either an intervention or control village.

Results

There was a significant difference in the seroconversion rate among dogs in the intervention compared to the control villages, indicating that the collars had reduced the risk of dog seroconversion. Both the DAT seroconversion rate and LST conversion rate in children were lower in the intervention than in the control villages, suggesting that collars may have provided protection for children against the risk of zoonotic visceral leishmaniasis.

Background

In many foci of zoonotic visceral leishmaniasis (ZVL), domestic dogs are important reservoir hosts of the causative leishmania parasites transmitted by phlebotomine sandflies (Diptera: Psychodidae). Experimental trials carried out under field conditions in Iran have confirmed the efficacy of Scalibor dog collars in protecting against sandfly bites [1]. These results justified the same research team in carrying out a community-based intervention trial using deltamethrin-impregnated dog collars during the transmission season in the endemic zone of zoonotic visceral leishmaniasis in Kalaybar and Meshkin-Shahr in north-west Iran.

Materials and methods

Study area

The study area was 21 villages in the districts of Kalaybar and Meshkin-Shahr in north-west Iran.

Design

An intervention study was conducted whereby children aged 1-10 years were surveyed using the DAT and leishmanin skin test (LST). All domestic dogs were diagnosed by the DAT, while all stray dogs were poisoned with strychnine by the village health workers. Within each district, the trial villages were ranked and ordered in pairs with comparable transmission rates. Villages in each pair were randomly allocated as the intervention or control group, whereby a matched community design was constructed. Prior to each village survey, a meeting was held with local community leaders to explain the objectives and logistics of the intervention study.

For testing with the direct agglutination test (DAT), finger-prick blood samples were spotted on filter-paper and the DAT antigen was prepared by the standard method from Leishmania infantum promastigotes as described by El-Harith [2]. The cut-off point for positive infections
in humans was 1/1600, and in dogs was 1/800. Children with a high DAT response ≥1/3200 received particular clinical attention and were treated for VL.

The LST was derived from *L. major* and prepared at the Pasteur Institute of Iran. The volar surface of one forearm was injected intradermally with 0.1 ml antigen (at a concentration of 10^7 promastigotes/ml) and a control solution (0.1 ml sterile pyrogen-free PBS with 0.01% thimersal) injected into the arm. The LST response was measured 48 hours later, and an induration width of 5 mm or greater was taken as a positive response.

## Main study findings

### Pre-intervention baseline survey

The results of this survey confirmed the matching factor of the study design, as there was no significant difference between the control and intervention villages regarding the seroprevalence rates of VL in humans tested by the DAT and LST, as well as in dogs tested by the DAT.

In the 9 control villages, out of the 84% (1225/1493) of children tested by the DAT, 92 were positive (7.5%; range: 3.5-10.5%). In the 9 intervention villages, out of the 86% (1369/1593) of children tested, 107 were positive (7.8%; range: 5.4-11.7%).

Testing with the LST indicated that in control villages, 200/294 children were positive (21.6%; range: 12-26%), compared to 223/967 (23.1%; range: 12-27%) in the intervention villages.

The seroprevalence in dogs was 64/562 (11.4%; range: 8-13%) in the control villages compared to 56/426 (13.1%; range: 6-19%) in the intervention villages.

### Intervention

All 18 villages were revisited in March 2000 prior to the sandfly season (May-September), during which the Scalibor collars were applied to all domestic dogs aged at least 7 weeks in the intervention villages. Spare collars were left for village health workers for replacing lost ones or for application on puppies turning 7 weeks old. Health workers visited each household in all 18 villages every 2 weeks to keep a record of changes in the dog population or any problems with the collars.

### Post-intervention survey (December 2000-January 2001)

During the first year, the rate of dog loss and replacement was similar in the control and intervention villages, though it was significantly higher amongst the DAT-positive than the DAT-negative dogs. There was a significant difference in the seroconversion rate amongst dogs in the intervention villages compared to the control villages (3.2% and 6.2%, respectively), indicating that the collars had reduced the risk of dog seroconversion by 49%.

Regarding infection rates in children, there were comparable sero-recovery rates in the intervention and control villages (12.9% and 12.4%, respectively). However, the seroconversion rate in the intervention was lower than in the control villages (1.6% and 2.8%, respectively).

The LST conversion rate in the intervention villages was 1.2% compared to 2.0% in the control villages, suggesting that collars may have provided 40% protection for children against the risk of LST conversion.

The difference in conversion rates obtained by the DAT and LST could be explained by the fact that LST conversion has a longer latency than seroconversion. Therefore, it would be interesting to confirm the consistency between the results of both tests by another LST survey carried out 1 year later.

The results of the present study were considered sufficiently encouraging to justify a community-wide field trial of deltamethrin-impregnated dog collars against ZVL vector sandflies in Iran. This was the basis of another study funded by SGG2000 entitled: "A matched community intervention trial for reducing *Leishmania infantum* transmission in Iran with insecticide impregnated dog collars." Furthermore, a third study on the insecticide-impregnated dog collars was funded in 2001 entitled: "Could infections in wild canids limit the potential effectiveness of insecticide-impregnated dog collars for reducing *Leishmania infantum* transmission in Iran?"

### References


**Abstract**

This study aimed at evaluating the impact of deltamethrin-impregnated dog collars on Leishmania infantum transmission in the Islamic Republic of Iran. Eighteen villages were paired and matched by pre-intervention child prevalence of L. infantum infection. Within pairs, villages were assigned randomly as control or intervention. All domestic dogs in intervention villages were provided with collars throughout 2 transmission seasons. Children and dogs were surveyed on three consecutive occasions at yearly intervals, i.e. pre-intervention in 2000, year 1 post-intervention in 2001, and year 2 post-intervention in 2002. The incidence of L. infantum infection was measured by seroconversion and leishmanin skin test (LST) conversion in children, and by seroconversion in dogs. Significant protection against seroconversion was detected in both dogs (collars causing an average reduction in incidence of 66%) and children (reduction of 53%). The impact of dog collars was also evaluated on both bloodfeeding and mortality rates of local sandfly vectors.

A survey on the knowledge and attitudes of the community regarding visceral leishmaniasis was conducted in 50 villages in the endemic region. The questionnaire also included information about the role of domestic dogs in the region, details of care delivered by the dog owners to their dogs and their attitudes towards the collars.

**Results** Deltamethrin-impregnated dog collars significantly reduced the risk of L. infantum infection in dogs and hence its transmission to children. However, 2 years of collaring dogs failed to completely eliminate transmission in villages. This could be because transmission continues in sylvatic reservoirs that visit villages frequently (e.g. foxes and jackals). The trial described in this report only measured the impact on infection. In order to detect the impact of collars on the disease, their cost-effectiveness, and hence, to draw conclusions and recommendations about their use as a control measure for visceral leishmaniasis, a much larger population study was undertaken in a wide-scale trial that will end in 2004.

**Background**

This study was a follow-up of a small grants scheme project implemented in 1999. It was recommended to continue the study for another year in order to improve the accuracy of measurements of the epidemiological effectiveness of the intervention, to carry out sandfly surveys in each village for vector identification and test the entomological effectiveness of collars in field conditions, as well as to investigate the attitudes of the local populations concerning visceral leishmaniasis and dog collars.

**Materials and methods**

Eighteen villages were ordered into pairs matched by pre-intervention child prevalence of L. infantum infection. Within pairs, villages were assigned randomly as control or intervention. All domestic dogs in intervention villages were provided with Scalibor collars (Intervet®, Intervet International of Boxmeer, The Netherlands). The main sandfly species identified in Meshkin-Shahr and Kalayabar are Phlebotomus major and P. chinensis, followed by P. kandelaki.

**Conclusions and implications of the study**

- Deltamethrin-impregnated dog collars could clearly reduce the risk of L. infantum infection in dogs, and hence its transmission to children.
- There was inadequate knowledge of the population regarding the role played by the vector or reservoirs regarding disease transmission. On the other hand, there was a high acceptability for using dog collars and evidence favouring the sustainability of their use in the future.
- The main sandfly species identified in Meshkin-Shahr and Kalayabar are Phlebotomus major and P. chinensis, followed by P. kandelaki.
Impact of IIDG on children and dogs in the studied villages  The use of impregnated dog collars halved the risk of DAT conversion in children but had less impact on the LST conversion rate (21%). Greater (66%) protection was provided to dogs.

Post-intervention data in children  During the 2-year trial, a total of 115 DAT conversions was detected amongst the 4324 follow-up tests in initially DAT-negative children. There was a significant effect of collars on DAT conversion per year. A total of 66 LST conversions were detected among the 2562 follow-up tests in initially LST-negative children. A significant effect of treatment on the risk of LST conversion per year was reported.

Post-intervention data in dogs  During the 2-year trial, a total of 67 DAT conversions were detected amongst the 1622 follow-up tests in initially DAT-negative dogs. There was a significant effect of treatment on the risk of DAT conversion per year. The impact in the second year (71% protection) was greater than in the first year (54% protection), suggesting a cumulative effect.

KAP study  Out of 1872 villagers interviewed, 727 were dog owners (39%). The survey was conducted among these dog owners indicate that they are willing to spend considerable money for dog care, that 66% of dogs are in good health, but that only 22% were vaccinated.

Many observations favoured the potential for sustained collar use throughout the transmission season such as: the low dog population turnover rate, the low rate of collar loss, the fact that most guard dogs (71%) nearly always stay at home, the fact that most dogs have long hair (97%) which presumably helps the effectiveness of the collars, the fact that most dogs already use metal collars (70%) for protection against bites by other dogs or wolves, and that dogs are not irritated by the new Scalibor collars.

Only 55% had heard of kala-azar and 39% believed that kala-azar was related to dogs. Half the respondents believed that children got sick with kala-azar due to the bite of an insect, but only 10% mentioned sandflies specifically. Most of the dog owners were satisfied with dog collars but only 42% thought that they could prevent kala-azar. Respondents have seen stray dogs and foxes in their village, and 42% saw jackals and wolves. Almost all villagers recognized that people get sick from dogs, with the most common disease mentioned being rabies. They also reported that their dogs suffer from fleas or ticks. So the collars should be helpful for dog health irrespective of their impact on sandflies. The majority of dog owners were satisfied with dog collars. However, only 42% of respondents thought that collars could
Abstract
The AMRAD-ICT Filariasis Test (ICT-Fil) is a new, rapid-format card test for the detection of bancroftian antigenaemia in human blood. The performance of the test was evaluated under field conditions in Egypt among 1813 subjects residing in known filariasis endemic foci and 102 participants from non-endemic filariasis areas. Participants from endemic foci were tested for microfilaraemia (thick smear and membrane filtration) and serum antigenaemia (ELISA).

Results
The infection rates detected were 2.8% by thick smear, 3.5% by membrane filtration, 8.8% by ELISA and 9.0% by ICT-Fil. The card test detected antigenaemia in 98.0% and 95.3% of microfilaraemia carriers testing positive by thick smear and blood filtration, respectively. Participants from non-endemic foci were ICT-Fil-negative. Identical results were obtained for 173 out of 184 participants (94%) from endemic foci tested by the serum and whole blood ICT-Fil versions.

Publications

Background
Nocturnally periodic bancroftian filariasis is endemic in several foci in the Nile delta in Egypt where it represents a major public health problem. Routine surveillance of the disease relies on detection of microfilaraemia in thick blood films, but this method was proved to be insensitive and impractical due to the necessity of blood collection at night. The research group has shown that filarial antigen detection by a monoclonal (AD12) antibody-based, enzyme-linked immunosorbent assay (ELISA) is sensitive and specific for the diagnosis of active *Wuchereria bancrofti* infections [1]. This method allowed for the detection of parasite antigenaemia in finger-prick blood collected during the day. However, the system required sophisticated equipment and cannot be performed directly in the field. Recently, a filariasis card-kit was developed by the Immunochromatographic Diagnostic Tests (AMRAD ICT) Company, Balgowlah, Australia. This ICT Filariasis (ICT-Fil) test is an antigen capture format that uses the AD12 monoclonal antibody and a polyclonal antibody attached to colloidal gold to detect filarial antigens in sera or plasma. A new version of the filariasis card-kit test was then developed that was based on the detection of circulating *W. bancrofti* antigens in finger-prick blood directly in the field. This study was conducted aiming at evaluating the performance of the whole blood ICT-Fil card test under field conditions in filariasis-endemic areas.

Conclusions and implications of the study
The ICT-Fil test is highly sensitive and specific, with excellent negative predictive values but low positive predictive values as it detects antigenaemia in amicrofilaraemic individuals.

The overall filaria prevalence rates for the ICT-Fil test and the monoclonal antibody-based ELISA test were fairly close (8.8% and 9.0%, respectively)

The test has tremendous technical and practical advantages over microfilaria detection for routine surveys. It can be performed during the day and results are obtained quickly, directly in the field. The test does not require any laboratory facilities or experienced microscopists and can be easily read by individuals in rural clinic setting.

This test could play a major role in the assessment of filariasis endemicity in affected communities, which is the basis of successful implementation of control activities.
participants (9 years and over) were selected from a longitudinal study designed to follow a population subset, based on index children from Tahoria middle school which serves nearby villages. After obtaining demographic information, household members were physically examined. Four diagnostic tests were performed on a total of 1813 subjects from endemic foci (924 females and 888 males). Another 102 control subjects from a non-endemic area in Cairo were tested by the ICT-Fil test and ELISA.

The ICT-Fil test was carried out using a finger-prick of blood drawn onto the card and the results visually read after 15 minutes by 3 examiners, then confirmed after being left overnight. Individuals were considered positive only upon agreement between the 3 readings.

Blood samples were obtained at night. Thick films and membrane filters were Giemsa-stained and microscopically examined for the presence of microfilariae.

Filarial antigen was detected in plasma by AD12 monoclonal antibody-based ELISA [1]. The evaluation was based on rigorous comparison tests; membrane filtration of blood collected during the peak hours of microfilaraemia was used as the gold standard, plus microfilarial detection by thick blood smears, as this is the method currently used for routine surveys.

### Main study findings

Of the 1813 subjects studied by the 4 tests, 64 (3.5%) had microfilaremia by membrane filtration. The mean ± SD of microfilaria (MF) count in these subjects was 123 ± 158 MF/ml (range 1-755, median 81 MF/ml). Of these, 50 (67.1%) were microfilaremic by thick smears, and 61 (95.3%) had positive ICT-Fil test. In addition, one case had 1 MF by thick film examination and positive ICT-Fil tets, but was negative for microfilaraemia by membrane filtration. The mean ± SD MF count by thick smears was 13 ± 12 MF /50 ul (range 1-54, median 9MF/50ul).

The ICT-Fil test successfully identified 98% of microfilaraemic participants detected by thick smear and 95.3% identified by membrane filtration, whereas control subjects were uniformly negative. These results showed that the ICT-Fil test is highly sensitive and specific, and is highly recommended as a replacement for the microfilaria detection tests currently used in the routine surveillance of bancroftian filariasis. The test had excellent negative predictive values (99.9% and 99.8%), but its positive predictive values were low (30.7% and 37.4%) when compared to microfilaria detection by thick smear and membrane filtration, respectively. This could be explained by the fact that the ICT-Fil test detects antigenaemia in amicrofilaraemic individuals, the latter being actually infected with *W. bancrofti*.

Although the overall filaria prevalence rates for the ICT-fil test and the AD12-ELISA were fairly close (8.8% and 9.0%, respectively), these tests did not detect the same antigen-positive endemic normal individuals. This was attributed to the different detection systems use in these assays. In this study, however, antigen levels were not quantitatively measured.

The whole blood version of the ICT-Fil test showed slightly higher sensitivity than the serum version. Twenty subjects (1.1%) in the study population had clinical filariasis: 11 with lymphedema, 6 with hydrocele and 3 with elephantiasis. All were amicrofilaraemic, ICT-Fil and ELISA negative, except for two cases with hydrocele that had MF (4 and 65 MF/ml, respectively) and were ICT-Fil and ELISA positive.

### Conclusions

This second generation whole blood ICT-Fil test has tremendous technical and practical advantages over microfilaria detection tests currently used for routine surveys. It can be performed during the day and results are obtained quickly, directly in the field. The test does not require any laboratory facilities or experienced microscopists. Because less than 1% of the ICT-Fil cards were questionnable, this indicates that, given adequate training, the test can be performed and easily read by individuals in rural clinic settings.

### References

Abstract

The present study was conducted in 8 laboratories in Khartoum State during the period September 1995–December 1996 to evaluate the accuracy of diagnosis of malaria in selected public and private laboratories. All patients referred for fever were examined. A blood sample was taken and 2 slides were made for each patient; one for routine blood film and the other was processed using the standard Giemsa stain as a reference technique in the National Health Laboratories.

Results

The standard Giemsa technique showed that 13% of cases with fever were positive for malaria. *Plasmodium falciparum* was the main species identified (96.2%), and the remaining proportion was *P. vivax*. The sensitivity of the light microscope ranged from 50.0% in some laboratories to up to 75.0% in others. Similarly, its specificity ranged from 52.6% up to 95.7%. These results indicate that the performance of the field laboratories was strikingly variable. The low sensitivity of the light microscope reveals that many positive cases will remain undiagnosed. And the low specificity in other laboratories reveals that a considerable percentage of individuals will be wrongly diagnosed as having malaria. In 4 laboratories, the results were absolutely unreliable. Suboptimal performance was attributed to the high workload and poor supervision.

Conclusion

This study emphasizes the need to direct limited health care resources to the provision of laboratory facilities, continuing medical and technical education of health professionals, and to organizing training workshops for the technical personnel.

Background

The prevalence of malaria in Sudan remains constantly high due to the presence of favourable environmental conditions and the progressive expansion and cultivation of agricultural land. Since light microscopy is the most reliable diagnostic method of parasitaemia [1], prompt diagnosis of malaria cases is dependent upon the accuracy of laboratory investigations, provided that results could be rapidly communicated to the healthcare providers. The aim of this study was to evaluate the accuracy of diagnosis and staining techniques of malaria in public and private laboratories.

Materials and methods

Eight public and private laboratories were selected from the 3 administrative Khartoum areas. The personnel of these laboratories were briefed about the study. All patients referred for fever were examined. A blood sample was taken from each patient and 2 slides made, one for routine blood film (Field stain) [2] and the other the processed using the standard Giemsa stain as a reference technique (Reference stain) in the National Health Laboratories (NHL).

A patient was considered negative if no parasites were seen in 100 microscopic fields of each slide. After examination and identification of the parasite species and stage, the parasite density was determined by counting the number of parasites against 300 leucocytes i.e the number of parasites per microliter of blood in a thick film is counted in relation to a standard number of leucocytes [3].

All the slides were examined by the principal investigator. Then, all positive
slides and 20% of negative slides were re-examined (blindly cross-checked) by a senior haematologist. The two sets of results were then compared and in case of discrepancy, the final diagnosis was established after a third examination by a co-investigator. Data collected from the 8 laboratories was recorded in special data collection forms. Field stain results obtained by the light microscope were then compared to the results of the reference stain obtained from the NHL.

Statistical analysis The validity of diagnosing malaria with the light microscope was computed using the SPSS statistical package.

Main study findings
The standard Giemsa technique indicated that 13% of patients presenting with fever were positive for malaria. *P. falciparum* was the main species identified (96.2%), and the remaining proportion was *P. vivax*.

The sensitivity of the light microscope ranged from 50.0% in some laboratories to up to 75.0% in others. Similarly, its specificity ranged from 52.63% up to 95.65%. These results indicate that the performance of the field laboratories was strikingly variable. The low sensitivity of the light microscope reveals that many positive cases will remain undiagnosed (high frequency of false-negative cases). This low specificity also reveals that a considerable percentage of individuals will be wrongly diagnosed as having malaria (false positive). In 4 laboratories, the results were absolutely unreliable. This suboptimal performance was attributed to the high workload and poor supervision. Individual technicians do not accurately follow the steps of the staining technique. They never adjust their time according to the diminishing concentration of stain throughout the day. The same amount of diluted stain might be used for several days without filtration. In other instances, they use water, which might be contaminated, instead of buffer. As a result, the chromatin of the malaria parasites is seldom poorly differentiated and the microscopic field contains a lot of micro-organisms and dirt. As a rule, 100 microscopic fields of each slide should be examined. This is rarely being done. Usually, after coming across the first few parasites, the technicians declare the slide positive and decide which species is involved. On the other hand, if the slide is too poor to be examined, because the blood has been washed or otherwise destroyed and no parasites are in sight, the slide is always declared negative. These laboratory errors, in addition to the malfunctioning of the available light microscopes, indicate that the diagnosis of malaria should be determined with Giemsa rather than with Field stain.

Conclusions and recommendations
The results of this study indicate the necessity to abstain from the use of Field stain for malaria diagnosis. The study also emphasizes the need to direct the limited health care resources to the provision of laboratory facilities such as equipment and the regular supply of reagents. Continuing medical and technical education of health professionals, together with training and tight supervision of the technical personnel, is warranted. The importance of establishing a quality assurance system is also emphasized.

References


Abstract

Malaria transmission in Gezira State, an endemic area in Sudan, is mainly seasonal, with two annual peaks in October and January. Questionnaire surveys were conducted for a 4-week period of October 1995. A sample of 400 households (3062 persons), including 200 rural and 200 urban households, was studied. Malaria management was assessed in terms of diagnosis, types of antimalarial drugs used, self-medication and compliance.

Results

One-quarter of the rural and 35.6% of the urban population received at least one course of antimalarial drugs during the 4 weeks. Diagnosis was confirmed microscopically in 81.7% and 34.3% of treated persons in urban and rural communities, respectively. Although chloroquine was the most frequently used antimalarial drug in both communities, there were significant differences in the pattern of its utilization in the two studied communities. Overuse of chloroquine injection was notable in rural patients and in those managed by paramedical health workers. By contrast, oral chloroquine was more commonly prescribed by medical doctors or taken as self-medication in the urban community. Self-prescribed medication was more common in the rural than in the urban population (40.2% and 23.9%, respectively). Compliance with the standard therapeutic doses was poorest with quinine and best with sulfadoxine/pyrimethamine.

Conclusion

Improving antimalarial treatment practices requires the dissemination of recent therapeutic guidelines and national health policies, improvement of diagnostic services, and health education. It is also concluded that self-medication is an important component of malaria management that needs to be streamlined in endemic areas.

Background

Rational use of antimalarial drugs is a key element for the control of the disease and for coping with the emergence and spread of drug resistance. In communities with limited access to health care facilities, most patients resort to self-medication [1]. Over-the-counter use of drugs and non-compliance are major factors leading to drug resistance. In areas where malaria is endemic, the home is the setting where diagnosis and treatment of most cases of malaria take place [2]. Early diagnosis and adequate health care eventually affect the prognosis of the disease. In Sudan, strains of Plasmodium falciparum resistant to chloroquine are already prevalent [3] and resistance to pyrimethamine/sulfadoxine has been reported [4], attributed mainly to irrational use of antimalarial drugs such as chloroquine, quinine, pyrimethamine/sulfadoxine and mefloquine. The present study was conducted to evaluate the pattern of use of antimalarial drugs at the community level.

Materials and methods

Gezira State is the main crop-producing agricultural area in Sudan. Malaria due
to *Plasmodium falciparum* is highly prevalent, with a seasonal peak in October following the rainy season. Four communities were involved in the study, 2 urban and 2 rural, with different socioeconomic levels and accessibility to healthcare facilities. The urban communities were residents of 2 blocks in Wad-Medani town, the capital of Gezira State, while the rural communities were residents of Ganneb and Shukkaba villages, south of Wad-Medani. Random selection of the households within each locality was performed. Household occupants were then interviewed by trained field workers for a 4-week period during October 1995, using a structured questionnaire.

### Main study findings

There was a marked discrepancy between urban and rural communities regarding laboratory diagnosis of cases. The rate of utilization of these facilities was higher in urban areas, indicating good awareness of the disease and the tendency to obtain a confirmed laboratory diagnosis, even among those who did not seek medical consultation. The scenario of empirical diagnosis and treatment of fever with antimalarials in the rural population is comparable with other endemic countries.

There was over-the-counter use of antimalarial drugs. However, self-medication could be beneficial if streamlined by the appropriate instruction of health workers in health facilities. In the present study, abuse of injections was more notable in cases treated by paramedical personnel than by medical doctors. It is interesting to note that self-medication in rural areas, where most of the health care is delivered by paramedical personnel, is characterized by greater use of injections. Conversely self-medication in urban areas, where most patients seek medical consultation, was predominantly by oral medications. It seems that patients are influenced by the practices of their attending clinicians. In this area, the paramedical personnel are generally underpaid and their income depends, to a great extent, on dressing wounds and giving injections, which explains the higher frequency of injections in rural areas. However, cultural beliefs that injections are more potent than tablets cannot be ruled out. Overuse of injections is an unnecessary burden to the meagre health resources. At the time of this survey, a course of oral chloroquine for an adult in Sudan cost the equivalent of US$ 0.45, whereas a course of 5 chloroquine injections cost at least US$ 3.06, including the price of the drugs, fees for the nurse giving the injections and expenses for the transportation to the health facility where the injections are given. Therefore, the unnecessary use of injections increases the cost of treatment by at least 500%.

Compliance with therapeutic doses of antimalarial drugs was poor with quinine, but optimal with chloroquine owing to the Blue Nile Health Project, an extensive control programme undertaken in Gezira during the period 1979-1989. This programme has raised the general public awareness of malaria in the Gezira area.

Of the treated urban population 56.6%, a high proportion, had taken antimalarial drugs within the 2 weeks prior to the survey and 16.0% repeated treatment during the 4 weeks of observation. The repeated use of drugs by the same patient may be explained by many factors: treatment failure due to low therapeutic efficacy of antimalarials, use of sub-therapeutic doses, and erroneous primary diagnosis. Finally, the results of this study indicate the need to assess the validity of laboratory diagnosis and therapeutic efficacy of antimalarials in the study area.

### Recommendations

1. Improve the existing laboratory diagnostic services, and extend these services to rural communities.
2. Test the therapeutic efficacy of antimalarial drugs and communicate this information to health policymakers.
3. Educate drug dispensers on the recent therapeutic guidelines, with special emphasis on self-medication. Rational use of injections is also recommended.

### References

Abstract
This study was conducted to assess the therapeutic efficacy of chloroquine against Plasmodium falciparum malaria in Sudan. An in vivo test was implemented in 5 sentinel posts in areas of unstable malaria during the transmission season. A standard dose of oral chloroquine was administered to a random sample of patients with uncomplicated falciparum malaria attending primary health care units and were followed-up for clinical and parasitological response for 14 days. Designations of “early treatment failure”, “late treatment failure” and “adequate response” were based on clinical and parasitological criteria. Data analysis for prevalence of resistance was done for each individual sentinel post, using 2-stage Lot Quality Assurance Sampling.

Results
At 95% confidence level and 80% power, the prevalence of chloroquine resistance was found to be 25% in all 5 posts.

Conclusion
It is concluded that the protocol was simple and easily applicable and could be the basis of future sentinel posts for continuous monitoring of malaria drug resistance for the whole country.

Background
The ultimate goal of malaria treatment policies is to ensure prompt, effective and safe treatment of malaria, which is one of the basic technical elements of the WHO Global Malaria Control Strategy. The standard in vivo test for the assessment of the therapeutic efficacy of antimalarial drugs was developed and standardized by the WHO. However, the test in its original form was too demanding to be applied as a routine field test in areas with limited resources. Accordingly, in certain studies, blood film examinations were performed on 3 occasions only instead of daily. This study was undertaken to evaluate the therapeutic efficacy of antimalarial drugs in Sudan.

Materials and methods
Study area
Five sentinel posts were selected from northern and central Sudan. Selection of these posts was based on the availability of well-equipped laboratories, the possibility of cross checking the results, the accessibility to hospitals for case referral and adequate community participation. The selected posts were health centres in Wad Medani, Kenana, Kassala, El-Obied and Dilling.

Clinical examination
The clinical examination of symptomatic patients was confirmed with laboratory investigations. Patients were instructed to present themselves at the clinic during days 0-14 in case of development of side-effects. The inclusion criteria were a history of fever during the past 48 hours, mono-infection with uncomplicated P. falciparum, and adherence to follow-up visits. Informed consent was obtained from the patients or the child’s caregiver. Individuals with other febrile diseases

Conclusions and implications of the study
- The proportion of clinical failures was unacceptably high in all sentinel posts. This emphasizes the need for revision of the current malaria treatment policies in Sudan, which consider chloroquine as the first-line drug for treatment of uncomplicated P. falciparum malaria.
- The relatively high prevalence of drug resistance in 2 out of the 5 posts was mainly attributed to the spread of resistant strains from neighbouring African countries as well as to displaced populations from southern Sudan.
- Both early and late treatment failures were significantly higher in children than in adults. Moreover, children had a significantly higher parasite count compared to adults. This could reflect lower immunity in children. It also indicates that assessment of therapeutic failure should consider children as a subgroup with a higher risk of therapeutic failure.
- The study was conducted under different epidemiological conditions (inter-area comparison), which makes it feasible in other African countries. It is finally recommended to develop other sentinel posts as an initial step for a nationwide plan for malaria control.
were excluded from the study.

**Laboratory diagnosis** Preparation and staining of the blood slides were performed according to the standard WHO procedures.

**Treatment** All cases with uncomplicated malaria were treated with oral chloroquine tablets in a 3-day course with the following doses: Day 0: 10 mg/kg, Day 1: 10 mg/kg and Day 2: 5mg/kg body weight. For children, the appropriate number and fraction of tablet (to the nearest 1/4 of a tablet) were crushed and administered with water in a spoon.

Patients recording treatment failure were treated with either appropriate doses of sulfadoxine/pyrimethamine or with quinine.

The administration of paracetamol on D-0, D-1 and D-2 was permitted when the patient's condition warranted such medication.

**Follow-up** For each patient there was a form on which clinicians were asked to record any prescribed medications during the next 14 days.

**Therapeutic response** Three categories of response to therapy were considered: adequate response (AR), early treatment failure (ETF), and late treatment failure (LTF). These are defined as follows:

- **AR:** Parasitaemia on D-3 < 25% count of D-0 and no parasitaemia on D-7 and D-14.
- **ETF:** Parasite count on D-3 (25% of count on D-0, or development of danger signs or other criteria of severe malaria OR complicated malaria on D-3 or before, in the presence of parasitaemia.
- **LTF:** Parasite counts on D-3 < 25% of count on D-0, and parasites present on scheduled visits on D-7 or D-14 OR unscheduled presentation after D-3 due to the development of danger signs or other signs of severe and complicated malaria or failure to improve, in the presence of parasitaemia.

Individuals with ETF or LTF were given alternative treatment.

The laboratory equipment, supplies and the microscopic examination of slides were subjected to quality control measures.

The sample size was estimated using the Lot Quality Assurance Sampling (LQAS) method.

### Main study findings

Out of a total of 242 patients initially enrolled, there were 9 (3.7%) lost to follow-up.

The proportion of treatment failures in all posts was not significantly less than 25% with a higher proportion in Dilling (72%) and Kassala (77%). The proportion of clinical failures was unacceptably high in all the sentinel posts covered. This calls for revision of current malaria treatment policies in Sudan, which still consider chloroquine as the first-line drug for treatment of uncomplicated *Plasmodium falciparum* malaria.

The relatively high prevalence of drug resistance in Kassala was expected because this post is close to the eastern borders of Sudan where importation of drug-resistant strains from neighbouring Eritrea had long been suspected. The high prevalence of resistance in Dilling was probably due to the sample population that included displaced populations from southern Sudan where chloroquine-resistant *P. falciparum* is suspected to have been imported though continuous population movements from neighbouring African states of Uganda, Kenya and Congo, where drug resistance has been reported.

Both early and late treatment failures were significantly higher in children than in adults, with 61.2% of children recording treatment failure compared to 33.9% of adults. Moreover, children had a significantly higher parasite count compared to adults. This could reflect lower immunity in children. More importantly it indicates that even in areas of low transmission, the assessment of therapeutic failures should concentrate on children as a subgroup with a higher risk of therapeutic failure. Several reports demonstrated the widespread chloroquine resistance of *P. falciparum* in Sudan. However, the present study assessed both the clinical and parasitological responses of the patient while previous studies considered only the clinical response in evaluating the overall response to treatment. This led to the designation of “treatment success” in cases that show clinical improvement in spite of persistent parasitaemia.

Furthermore, the study was conducted under different epidemiological conditions (inter-area comparison), which makes it feasible for other African countries. This study also provides the essential information needed for policy-makers. It gives a fairly accurate estimate of chloroquine resistance, which should influence treatment policies in the studied area. It is finally recommended to develop other sentinel posts as an initial step for a nationwide plan for malaria control.
Abstract
Malaria in Saudi Arabia is localized to the southern and south-western parts of the country, with the highest frequency of cases reported from Jezan and Asir regions. A recent epidemic in Jezan region was associated with high mortality and severe morbidity suspected to be related to the emergence of chloroquine-resistant malaria falciparum. This study was undertaken to assess the efficacy of chloroquine therapy for *P. falciparum* malaria, in Al-Khishil, Jezan, south-western Saudi Arabia. A total of 291 laboratory-confirmed malaria patients were enrolled in the study. The patients were given a standard 3-day regimen of chloroquine and were examined clinically and parasitologically for malaria on the fourth day. Positive cases were given sulfadoxine pyrimethamine and checked for malaria parasites on the seventh day of treatment.

Results
The cure rate of malaria with the standard chloroquine therapy was 87.6%. Chloroquine-resistant patients were successfully treated with sulfadoxine pyrimethamine.

Conclusion
Chloroquine is still an efficacious drug for the treatment of malaria in the region. The treatment failure is acceptable (12.4%) compared to other parts of the world. However, the level of drug resistance should be carefully monitored and analysed in relation to changing dynamics of malaria transmission in the region.

Background
Malaria is endemic in Asir and Jezan Lowlands ("Tihama") region in south-western Saudi Arabia. There are 2 yearly peaks of transmission, both associated with rainy seasons, one in winter (December to April) and the other in summer (June to July). In 1998, an epidemic was reported from Asir and Jezan regions. In the next winter season, the disease became highly endemic in Jezan whilst declining significantly in Asir. It was associated with high mortality and severe morbidity in Jezan, whereby the emergence of chloroquine-resistant malaria falciparum was suspected. This study was undertaken to assess the efficacy of a standard regimen of chloroquine (CQ) therapy in regard to *Plasmodium falciparum* malaria, in Al-Khishil, Jezan, south-western Saudi Arabia.

Materials and methods
Study setting Based on the records of the regional Ministry of Health, a primary health care centre recording a high prevalence of malaria transmission in Al-Khishil in Jezan region was selected.

Study population The study population consisted of individuals with clinical manifestations and confirmed laboratory diagnosis of malaria, while patients suffering from severe or complicated malaria were excluded from the study.

Study design All patients enrolled in the study were examined clinically and parasitologically on presentation and were then given a standard, 3-day regimen of chloroquine.
oral CQ (i.e. 10, 10 and 5 mg CQ base/kg body weight on days 0, 1 and 2, respectively).

Fingerprick blood samples were collected on days 0, 3 and 7, used to make thick smears, stained with 10% Giemsa stain for 10 min, and then checked for malaria parasites. The level of parasitaemia (parasites/µl blood) was estimated by counting parasites against 500 leucocytes and assuming that each patient had 8000 leucocytes / µl blood.

Each patient was assessed clinically on days 0, 3, 7 and day 14. During the complete physical examination given on each of these occasions, special attention was given to the malaria-attributable symptoms of fever, rigors, vomiting, diarrhoea, dehydration, cough and jaundice. Body temperature, taken as a marker of illness or clinical cure, was recorded carefully over 3 min, on days 0, 3, 7 and 14, with the standard, graduated, Mediturf thermometer (Great New Life, Suzhou, China) used routinely by the primary healthcare facilities in the region; this thermometer has a temperature range of 35-42°C graduated in intervals of 0.1 °C.

Any individual found to have a malarial infection on day 3 was then given replacement therapy- a single dose of Fansidar (Roche), representing 25 mg sulfadoxine and 1.25 mg pyrimethamine/kg. The protocol was carried out according to the WHO guidelines for Good Clinical Practice [1].

Data analysis The demographic, clinical, parasitological and treatment data collected were recorded and analysed using version 7.5 of the SPSS software package (SPSS Inc., Chicago, IL).

Main study findings A total of 291 patients, out of 350 parasitologically positive clinical cases, completed the study. Chloroquine was found to be a safe and efficacious drug in the treatment of malaria in this region as 87.6% of treated patients achieved a clinical and parasitological cure with the standard regimen of chloroquine therapy.

Treatment failure accounted for 12.4%. The non-responders had clinical symptoms and were parasitologically positive on the fourth day post treatment. All patients who failed to respond to chloroquine therapy, responded well to a single standard regimen of sulfadoxine/pyrimethamine. The lack of response to chloroquine correlated significantly with high body temperature on initial presentation. The mean temperature of non-responders was 39.1 °C ± 0.14 °C compared to 38.7 °C ± 0.05 °C in responders. On the other hand, there was no correlation between the non-response to chloroquine therapy and the age of the patient or the degree of parasitaemia at initial presentation. The factors underlying the treatment failure could be related to the parasite and/or host factors. The possibility of cross-border importation of resistant strains from neighbouring countries such as Yemen was also highlighted.

Conclusions and recommendations This study confirms the efficacy of chloroquine for the initial management of malaria. Sulfadoxine/pyrimethamine is a safe and efficacious antimalarial for the management of patients not responding to chloroquine in this region.

The level of treatment failure was within an acceptable range compared to other parts of the world. However, the emergence of drug resistance should be carefully monitored and analysed in relation to the changing dynamics of malaria transmission in the region.

Abstract
This study aimed at exploring the current epidemiological profile of malaria in the post-war period. Fifty major hospitals were randomly selected from all private hospitals in Lebanon. Laboratory records were reviewed for diagnosed cases of malaria during a 3-year period (1 September 1994 to 31 August 1997). Details related to the frequency of requesting malaria smears, diagnostic techniques and case reporting were recorded.

Results
There was a high frequency of requesting malaria smears, mainly performed as a routine investigation for travelling purposes. A total of 228 malaria cases were detected during the study period (1.3% of requested smears). Among the minority of cases subjected to species identification, Plasmodium falciparum proved to be the predominant type (88.9%), while Plasmodium vivax was rarely identified (5.6%). Neither the laboratory investigations nor the case reporting techniques were standardized over the country.

Conclusion
It was suggested that the resurgence of malaria in Lebanon in recent years could be attributed to emigrants from Africa due to the high relative frequency of P. falciparum compared to P. vivax, as the latter proved to be of an endogenous origin. Diligence in case detection, and better efficiency and standardization of the case reporting system and laboratory investigations are finally recommended.

Background
During the war, there were no statistics on diagnosed cases of malaria in Lebanon, aside from the interrupted and incomplete case reporting to the malaria office, Ministry of Public Health. Furthermore, due to improving methods of transportation, ease of travel, and climatic changes, the WHO has warned against the spread of malaria around the world, especially in the 'Middle East'. These factors prompted researchers in Lebanon to study the current epidemiological profile of malaria through conducting a survey on laboratory records.

Materials and methods
As a result of the collapse of public health services after the war, the health care system in Lebanon became confined to private health services partially funded by the Ministry of Public Health. Fifty major hospitals were randomly selected from all private hospitals distributed over the 5 Lebanese Governorates. Letters were sent to the administrators and laboratory physicians in the selected hospitals and were contacted by phone. Interviewers visited the laboratories of these hospitals and reviewed laboratory records for diagnosed cases of malaria during the 3-year period (1 September 1994 to 31 August 1997).

Data were not available for the whole study period in all laboratories. There were some missing months for 1994-1995, and only few missing months for 1996, while the records for 1997 were...
complete for all surveyed hospitals. Collected information included: the frequency of requests for malaria smears, the frequency of positive and negative cases, the technique of diagnosis and the method of reporting to the Ministry of Public Health. The information gathered from these laboratories was then compared to that provided by the Ministry of Public Health, and the rate of case reporting and its delay for each hospital were computed.

I Main study findings
There was a high frequency of requesting malaria smears per year (mean 5817 smears). These were mainly performed as a routine investigation for the completion of administrative requirements for travelling to or from the country. A total of 228 malaria cases were detected during the study period (1.3% of requested smears). The frequency of positive cases was 1.0%, 1.7% and 1.4% for the periods 1994-1995 (16 months), 1996 (12 months), and 1997 (8 months), respectively. The species of malaria was seldom identified (36/228); of those identified, *P. falciparum* was the predominant diagnosed type (88.9%), and only 2 cases were *P. vivax* (5.6%). The highest frequency of cases was in summer, which coincides with the return of Lebanese emigrants from Africa, and explains the predominance of *P. falciparum* malaria as imported from Africa. On the other hand, *P. vivax* cases were reported to be endogenous.

The thin blood film stained with Wright and Giemsa stain was the most commonly used method for diagnosis in 37 laboratories, May-Gruenwald (MCG) in 12 laboratories, and Field stain in 1. There were some variations within the laboratories regarding the incubation time of the different reagents. Thick blood films were only performed in 13 laboratories, with variable methods of preparation and staining. Blood films were primarily read by laboratory technicians and cross-checked by a specialized microbiologist.

Nevertheless, there was significant different in case reporting between the different laboratories. For the majority of hospitals this was carried out by a Ministry of Public Health employee who regularly visits these hospitals for data collection. On the other hand, some centres sent their positive results directly to the epidemiology unit of the Ministry of Public Health or the malaria office of the Ministry. Reporting seemed to improve over the study period, ranging from 34% in 1994-95, to 54% in 1997, with an average 9.15-days’ delay in reporting. Verification of available smears for reported cases was routinely done in the malaria office. However, there was some disagreement between the lists of reported cases at the malaria office and epidemiology unit in the Ministry of Public Health which improved with time due to better communication.

I Recommendations
As the resurgence of malaria in Lebanon in the recent years is mainly attributed to emigrants from Africa, diligent surveillance and chemoprophylaxis are considered one of the public health priorities for malaria control. The scarcity of preparation of thick films and the variability in staining procedure indicate the need for standardization of laboratory techniques according to the recent WHO guidelines for malaria diagnosis. Quality assurance for laboratory diagnosis of malaria is therefore recommended.

Furthermore, a need for a better efficiency and standardization of the case reporting system is also envisaged.

By exploring the epidemiological profile of malaria in the post-war period, a foundation is provided upon which to build a quality improvement programme using the parameters in this study as an initial framework.
Malaria Epidemiology

Abstract
This study aimed at analysing the epidemiological factors causing the persistence of malaria in Fayoum Governorate, the only remaining malaria focus in Egypt. The study took place in a village in Fayoum Governorate. The collected information included: house characteristics, presence of domestic animals, location of houses in relation to agricultural fields, water streams, swamps and brick factories. Monthly larval and adult mosquito surveys were carried out over a 1-year period. Relevant information on the breeding places and collected larvae or mosquitoes was recorded. Samples of the aquatic and semi-aquatic vegetation were collected and identified. Meteorological data collected included the average daily temperature, relative humidity, rainfall and wind speed during 1995-1996. Blood slides were prepared from persons with fever, from those who had a fever in the recent past or from those referred for blood examination for malaria parasites. Mass blood examination was performed by house-to-house visits.

Results
The factors causing persistence of malaria included: the hydraulic problem of subsoil water, swamps, favourable meteorological conditions, land excavation for the brick industry, house location in the vicinity of water streams, the shift from spraying cotton with insecticides to the use of perhormones, the use of pyrethrum and larviciding environmental and climatological conditions favouring malaria transmission throughout Egypt.

Conclusions and implications of the study
- An. sergenti and An. multicolor are the prevalent species in this area, with variable frequencies depending on the season and mean monthly temperature. The female indoor resting density was highest from October to December and the seasonal variation of Plasmodium falciparum extended from March to November.
- The dynamics of P. falciparum transmission in this district indicate a prevalence of hypoendemicity that is unstable and subject to epidemic exacerbation every 3-5 years.
- The persistence of malaria was attributed to the hydraulic problem of subsoil water, swamps, favourable meteorological conditions, land excavation, house locations near water collections, agricultural factors (diminished use of pesticides and increased cultivation of rice), in addition to the restriction of malaria health services to malaria units.

Background
Since 1946, there has been a dramatic decline in the malaria incidence in Egypt due to the successful application of DDT. By the late 1970s, the disease had become localized to 2 of the 5 districts of Fayoum Governorate. Despite the similar bio-environmental and climatological conditions favourable malaria transmission throughout Fayoum Governorate south-west of Cairo.

Study period: January–December 1996

Small Grants Scheme
(SGS) 1995 No. 22

Principal Investigator
Dr Hassan Kamel Bassioumy
High Institute of Public Health
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Alexandria, Egypt
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Epidemiology
Malaria
Egypt
Fayoum Governorate, Egypt

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Abstract
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the governorate, the disease was eradicated from 3 districts (Tamiya, Itsa and Ebshway) while still persisting in the 2 others (Sinnuris and Fayoum). This study sought to determine the epidemiological factors causing the persistence of malaria in Fayoum Governorate.

Materials and methods

Study area Since most reported malaria patients were residing in Kafr Fazara village in the central part of Sinnuris district, this village was selected as a study area. A census of all households was performed. The collected information included: house characteristics, presence of domestic animals, location of houses in relation to agricultural fields, and details of nearby water streams, swamps and brick factories.

Meteorological data Meteorological data collected included the average daily temperature, relative humidity, rainfall and wind speed during 1995-1996.

Larval surveys Monthly larval surveys were carried out over a 1-year period from randomly selected permanent water sources and sites with high larval density. Larvae were collected by the dipping and netting methods. Relevant information on breeding places and collected larvae was also recorded. Samples of the aquatic and semi-aquatic vegetation were collected and identified.

Monthly adult mosquito survey A monthly adult mosquito survey was performed over the year from randomly selected houses and animal sheds to obtain seasonal baseline data on malaria transmission. Information collected included vector determination, abdominal appearance, seasonal vector behaviour and abundance. Mosquitoes were collected in the early morning by the spray sheet collection method using pyrethrum solution and placed in small labelled boxes for counting and identification. Identification of the collected larvae and adult mosquitoes was carried out.

Parasitological survey Blood slides were prepared from villagers presenting with fever, with a recent history of fever, or from those referred for blood examination for malaria. Mass blood examination was performed by house-to-house visits and from the neighbours of positive patients. Duplicate slides were prepared from the peripheral blood by finger-pricking using disposable lancets. Giemsa stain was used as the reference technique. Parasite densities were recorded and classified into low class (up to 10 parasites per 10 fields) or high class (1 or more parasite per 1 field). Slides were primarily examined by technicians (field test), then cross-checked blindly by a senior microscopist (reference test 1), and the two results compared. All positive slides and 20% of randomly selected negatives slides were re-examined by the PI (reference level-2). This cross-checking at different levels increased the chances of detecting low-level parasitaemia.

Main study findings

The majority of the land is cultivated and most of the houses are located in the vicinity of water streams, are made of mud bricks and contain enclosed animal sheds. Other houses were modern, built with red bricks and concrete and were provided with electricity and water supply. The high level of subsoil water led to the formation of pools, seepage water collections and swamps. A big swamp (Abou-Naoura) was formed as a result of intensive excavation of agricultural land to obtain the clay necessary for the brick industry. In one of the houses, the bursting of subsoil water led to the formation of a small spring inside the house.

The results of the larval and adult mosquito surveys indicated that An. sergenti was the prevailing species followed by An. multicolor, while An. pharoensis was rarely encountered. An. sergenti recorded the highest percentage for gravidity in females while An. pharoensis recorded the highest percentage of fed mosquitoes.

There was a significant seasonal variation in the total number of collected larvae, which was influenced by the mean monthly temperature. The highest frequency of adult mosquitoes was recorded in animal sheds rather than bedrooms. The female indoor resting density showed significant seasonal variation, peaking from October to December.

Relating vector behaviour to meteorological data revealed that the transmission season of P. falciparum extends to more than 8 months per year, from the end of March to the end of November. The parasite density of the study population was 0.57%, with higher density in younger age groups. The dynamics of P. falciparum transmission in this district indicate a situation of prevalent hypoendemicity, but is unstable and subject to epidemic exacerbation every 3-5 years. The factors causing persistence of malaria include: the problem of subsoil water, swamps, favourable meteorological conditions, land excavation for the brick industry, location of houses in the vicinity of water streams, shifting from spraying cotton with insecticides to the use of pheromone that attract insect males, and increasing rice cultivation. Furthermore, the restriction of malaria health services to malaria units resulted in a decrease in the number of detected cases and a lack of reliable data concerning morbidity and mortality from the disease. Strengthening of the malaria control strategy as well as integrating the malaria control activities into the primary health care system are highly recommended.
**Malaria**

**Epidemiology**

**Pakistan**

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**Abstract**

The importance of *Anopheles stephensi* in the maintenance of malaria transmission in rural Pakistan needed further clarification. This project was conducted to clarify the role of *An. stephensi* in malaria transmission in areas near Lahore where *An. stephensi* is the dominant anopheline. Four villages were selected in Punjab province near Lahore. The population of the 4 villages was checked for the malaria parasite in the peripheral blood once per month from September 1995 to June 1996. Mosquito sampling was performed fortnightly from all villages. Humans and buffaloes were made available as bait once a month. Two types of dissections were carried out, immediate and late. For late dissections, mosquitoes were allowed to feed on infected blood and the gut and glands of mosquitoes were examined for the presence of the malaria parasite.

**Results**

Only 4 out of 1937 dissected mosquitoes were found positive at the oocyst stage. Delayed dissection of mosquitoes after feeding on infected blood revealed the development of oocysts and sporozoites on the 4th and 8th day, respectively, for *Plasmodium vivax* and on the 8th and 14-17th day, respectively, for *Plasmodium falciparum*.

**Conclusion**

The results suggest that *An. stephensi* could be a vector for *P. falciparum*. However, these results need to be confirmed with further studies due to the failure of previous attempts to find such an association in this area.

**Background**

*An. culicifacies* Gilles is recognized as the primary vector of rural malaria, [1] and is uniformly susceptible to malathion, the insecticide used for control by residual spraying, while in some areas of Punjab incipient resistance to this insecticide was reported [2]. Among the remaining 24 *Anopheles* species existing in Pakistan [3], only *Anopheles stephensi* Liston has been widely incriminated in malaria transmission and has also developed resistance to malathion [4]. This species was considered the primary vector of transmission of urban malaria in Karachi. The importance of *An. stephensi* in the maintenance of malaria transmission in rural Pakistan needed further clarification. In Punjab, comparative dissections of *An. culicifacies* and *An. stephensi* over an 18-month period failed to reveal sporozoite-positive *An. stephensi*, while positive *An. culicifacies* were detected from August to November [5]. The resistance of *An. stephensi* to malathion was also reported, and transmission has been successfully interrupted by the combination of malathion spraying and drug administration. This project was conducted with the aim of studying the role of *An. stephensi* in malaria transmission in areas near Lahore where *An. stephensi* is the dominant anopheline, and to study the entomological parameters of this species.

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**Role of Anopheles stephensi in transmission of malaria in Pakistan**

**Pakistan**

**Study period:**

September 1995–1996

**Small Grants Scheme**

(SGS) 1995 No. 23

**Principal Investigator**

Mrs Ghazala Toqir Nadeem

National Institute of Malaria Research and Training

Lahore, Pakistan

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**Conclusions and implications of the study**

- A negligible proportion (4/1937) of immediately dissected mosquitoes were proved to be positive at the oocyst stage.
- Delayed dissection of mosquitoes after feeding on infected blood revealed the development of oocysts and sporozoites on the 4th and 8th day, respectively, for *P. vivax*, and on the 8th and 14-17th day, respectively, for *P. falciparum*.
- Mosquitoes infected with *P. falciparum* develop a higher number of oocysts and sporozoites compared to *P. vivax*, suggesting a possible association between *An. stephensi* and *P. falciparum*. However, further confirmation of these results was recommended.

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**Materials and methods**

**Study area**

Four villages were selected in Punjab province near Lahore, 2 in district Kasur (Rohewal, Dera Islampura) and 2 in district Lahore (Billianwala and Mehdipur). The selection of villages was primarily based on elevated malaria...
incidence and on the abundance of *An. stephensi*.

**Methods** Maps and population censuses of the villages were completed along with house numbering.

**Malaria case detection** The population of the 4 villages was checked for malaria by blood testing for the parasite once per month from September 1995 to June 1996.

**Mosquito sampling** Collection of *An. stephensi* was performed fortnightly from all villages. Night biting collections from humans and buffaloes were made once each month. Field collected mosquitoes were kept in the insectary at constant temperature (27-28 °C) and humidity. Two types of dissections were carried out, immediate and late. For late dissections mosquitoes were allowed to feed on infected blood and to lay eggs, and were given blood meals from mice, and they were dissected 4 days after feeding. The gut and glands of the mosquitoes were examined for the presence of the malaria parasite.

**Major study findings**

Only 4 out of 1937 immediately dissected mosquitoes were proved to be positive at the oocyst stage. Reared *An. stephensi* dissected 4 days after feeding on infected blood revealed the development of oocysts of *P. vivax*, whereas sporozoites were seen on the 8th day after the blood meal in the salivary gland. In *P. falciparum*, the oocysts and sporozoites developed on the 8th and up to the 17th day, respectively, after the blood meal. Furthermore, there was a higher number of oocysts and sporozoites in *P. falciparum* compared to *P. vivax*.

When 43 mosquitoes infected with *P. vivax* were observed, it was noticed that 23 survived up to day 9 and none developed the parasite, while 20 mosquitoes developed oocysts on the 4th day (46.5%) whereas sporozoites were detected in only 1 mosquito on the 8th day (2.3%).

On the other hand, 31 out of 41 mosquitoes fed on blood infected with *P. falciparum* survived till the 17th day. Out of 31 dissected mosquitoes, 25 (80.6%) were positive for oocysts and 15 (48.4%) for sporozoites that were detected on the 14th day.

These results suggested that *An. stephensi* was associated with *P. falciparum*. However, it was concluded that these results should be taken with due caution and needed to be confirmed with further studies due to the failure of previous attempts to find such an association in this area.

**References**


Malaria in rural areas around Khartoum: the role of displaced people in the geographical distribution of different malaria species

Sudan
Dar El Salam, El Baraka and Jebel Awlia camps, rural Khartoum

Study period:
June 2001 – December 2002

Small Grants Scheme
(SGS) 2000 No. 49

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Abstract
This project was conducted in order to understand and evaluate the possible effect of displaced people in the transmission dynamics of the malaria parasite in rural areas around Khartoum. The study was carried out in 3 camps in Khartoum state: Dar Al Salam, El Baraka and Jebel Awlia. Duplicate thin and thick blood smears were collected from each suspected malaria case referred to the health facilities of the camps who were also interviewed to obtain information about socio-demographic variables and determinants of malaria infection. Malaria diagnosis and parasite species were confirmed by PCR. The level of parasitaemia in the area was also determined. An entomological survey was also carried out in the area to determine the vector species.

Results
The majority of inhabitants in the displaced camps belong to the Nuba, Dinka and Shuluk tribes, from Southern and Western Sudan.
Malaria infection was confirmed by microscopy in 73% of individuals referred to health facilities for suspected malaria, but the sensitivity of microscopic examination of blood was only 77%. Al Salam camp showed the highest infection rate of malaria, followed by Jebel Awlia and El Baraka camps. The level of parasitaemia in these areas ranged between 400 and 6960 parasites per microlitre of blood. Plasmodium falciparum infection constituted 63.3% of species identified, followed by mixed P. falciparum and P. malariae, and a few cases of P. malariae and mixed P. falciparum and P. vivax. No ovale cases were identified in this area.

The relative frequency of different malaria species did not vary significantly between the displaced people and those residing in the neighbouring villages. Mixed infections were significantly higher in people who stayed for more than 6 years, those with a negative history of malaria infection, and in people from Southern and Western tribes. Anopheles arabiensis is the only species in this area but no sporozoite-positive mosquitoes were identified.

Conclusion
Malaria infection does not vary significantly between people living inside or outside camps in rural areas around Khartoum.

Background
Accurate knowledge of the geographical distribution of the 4 Plasmodium species infecting humans is very crucial for designing proper control measures. These species differ greatly with respect to their biology, clinical manifestations and response to antimalarial drugs. When parasite levels are very low, the sensitivity of microscopy in detecting mixed infection is reduced. In these cases, PCR offers a great advantage. The detection of mixed infection is not only important for successful medical treatment, but also for ascertaining the relative frequency of each species and consequently its transmission potential. P. falciparum represents the main species found throughout Sudan. However, war, drought and population movement contributed to
the settlement of displaced people from different regions into Khartoum. It was expected that these new settlers had modified the relative frequency of various species, or introduced new ones. This was confirmed by recent reports of the increasing frequency of *P. vivax* in some villages around Khartoum.

Therefore, this project was conducted in order to understand and evaluate the possible effect of displaced people in the transmission dynamics of the malaria parasite in areas around Khartoum.

**Materials and methods**

The study was carried out in 3 selected camps of displaced people in Khartoum state: Dar Al Salam, El Baraka and Jebel Awlia. Al Salam and Jebel Awlia camps were established in 1991, while El Baraka camp was established in 1998.

The study area has a semi-arid climate, with a rainy season from August to October and a dry season from November to May. The mean temperature varies between 31.1 °C and 32.6 °C. Humidity is highest in August and September. Al Salam and El Baraka camps have a sandy soil, while Jebel Awlia has a clay soil. The vegetation in all camps consists of mainly semi-desert varieties.

A questionnaire was designed to collect sociodemographic and clinical information.

Duplicate thin and thick blood smears were collected from each suspected malaria case referred to the health facilities of the camps. Blood smears were stained with Giemsa according to standard methods.

The level of parasitaemia was also determined.

Filter paper blood samples were collected for subsequent DNA extraction, PCR analysis and identification of species using species-specific primers.

**Entomological survey**

Random collection of 875 indoor-resting adult mosquitoes was performed using an aspirator in unsprayed rooms in the 3 study sites. Identification of the Anopheles mosquito was accomplished as described by Gilles and De Meillon.

A group of 10 mosquitoes were crushed on 1 filter paper and kept at 4 °C before using them for DNA extraction and subsequently PCR.

**Main study findings**

The majority of inhabitants in the displaced camps belong to the Nuba, Dinka and Shuluk tribes, who migrated from southern and western Sudan, and a few tribes were from central and northern Sudan. These displaced people began settling in camps in 1991. The settlers are employed as labourers, soldiers, teachers, domestic servants, vegetable retailers and brick-makers. Some work as seasonal agricultural workers. Many of the males are employed outside the village and return to their homes late in the evening or on weekends.

Most of the houses in the study sites are made of mud or brick, and a few are constructed from wood or are simple shelters of old jute, plastic cartons or dried grass. The majority of the houses are not painted and have no windows.

Malaria infection was confirmed by microscopy in 107 out of 147 individuals referred to health facilities for suspected malaria (73%). On the other hand, malaria was confirmed by PCR in 94.5% of these individuals. Therefore, the sensitivity of microscopy in these areas was only 77%. Al Salam camp showed the highest infection rate of malaria, followed by Jebel Awlia and El Baraka, which was statistically significant by microscopy but not by PCR.

The level of parasitaemia in these areas ranged between 400 and 6960 parasites per microlitre of blood.

Using PCR, *P. falciparum* infection constituted 63.3% of the species identified, followed by mixed *P. falciparum* and *P. malariae* (25.9%), and a few cases of *P. malariae* (9.4%), and mixed *P. falciparum* and *P. vivax* (1.4%). No ovale cases were identified by PCR. Several false-positive results were obtained by microscopy which proved to be of low sensitivity in species identification.

The relative frequency of different malaria species did not vary significantly between the displaced people and those residing the neighbouring villages. This might be due to the fact that these villages are new residential areas established almost at the same time of the camps. Another factor may be that these camps and neighbouring villages have similar tribal, social and economic situations.

Mixed infections were significantly higher in people who stayed for more than 6 years, and for those with a negative history of malaria infection. In particular, the rate of mixed infection of *P. falciparum* and *P. malariae* varied significantly between tribes. In general, mixed infection was higher in people from southern and western tribes (50% and 47.1%, respectively). Few cases of mixed infection were detected among central tribes (2.9%), and none among the northern tribes.

The entomological survey showed that *A. arabiensis* is the only species in this area with no significant variation between the three studied camps regarding its indoor resting density. No sporozoite-positive mosquitoes were detected, suggesting low malaria transmission in rural areas around Khartoum.
Malaria

Knowledge Attitudes and Practice

Islamic Republic of Iran

**Abstract**

A cluster sample of 2168 housewives was randomly selected from the urban and rural areas of 5 districts, and interviewed using a questionnaire based on the Health Belief Model (HBM).

**Results**

The majority of subjects (67.5%) were illiterate. One-third of the respondents considered malaria as an important disease and 58.4% identified mosquitoes as vectors. There was poor knowledge regarding malaria; subjects recorded a total knowledge score of 15 and the mean scores in rural and urban areas were 5.5 and 4.7, respectively, with a significantly higher awareness regarding the disease among rural housewives. Similarly, the perceived susceptibility and severity of malaria were higher in rural residents. There was an association between the perceived role of mosquitoes in the transmission of the disease and the use of bednets although nets were primarily used to avoid mosquito stings. One-third of individuals used bednets. Indoor sleeping and fans were the main reasons for not using bednets and less than one-quarter of houses had window and door screens. Up to 73.8% of individuals received their first treatment from the local health house and health centre, and Behvarz was the main source of information pertaining to the disease, while the role of physicians was minimal.

**Conclusion**

This study showed that the individual perception of risk is the main determinant of adherence to malaria control procedures. Strengthening of the perception of risk by health education is therefore recommended.

**Activities achieved within the framework of the study**

A seminar was held whereby medical students were instructed regarding the socioeconomic level of the population, design of the study, sampling technique, items of the questionnaire and interviewing techniques.

**Background**

The Health Belief Model (HBM) is a psychological model that attempts to explain and predict health behaviour by focusing on attitudes and beliefs of individuals [1]. The key variables are the following [2]:

1. Perceived threat: entailing perceived susceptibility or perception of risk and perceived severity or perception of the seriousness of illness and possible consequences.
2. Perceived benefits: the effectiveness of strategies to control the disease.
3. Perceived barriers: the physical, psychological and financial factors undermining a negative health action.
4. Cues to action: events that motivate people to take an action.
5. Other demographic, socio-psychological and structural variables that influence health-related behaviour.
6. Self-efficacy: the beliefs in being able to successfully execute the behaviour required and produce the desired outcome.

According to the HBM, healthful behaviour is more likely if an individual feels susceptible to a condition, perceives a benefit to the behaviour while perceiving...

**Conclusions and implications of the study**

- This study uncovered marked deficiencies in the level of education delivered to housewives. However, increased knowledge about the benefits of bednets increased their usage. Health education programmes aimed at enhancing community participation in control strategies are warranted.
- The majority of individuals used bednets to avoid outdoor mosquito stings and abandon them during indoor sleeping. The health hazards of mosquitoes should therefore be communicated to the community.
- Physicians provided very low participation to the level of information delivered to villagers related to malaria. Only 50% of villagers had ever been taught by health care providers.
- A control programme could be successfully achieved if implemented by the village headman or party officials rather than by relying mainly on enhancing individuals’ knowledge of the perceived risks. This concept could be applied in countries with a comparable epidemiological profile.
relatively few barriers, and has cues to perform the action. Perceived severity and SES can also influence behaviour. Health beliefs are complex constructs with no reference standard for their assessment [3]. The components of the HBM hypothesize that behaviour is a function of 2 factors: the value an individual places on health (goal orientation) and the individual’s belief that specific preventive actions will achieve the goal. The HBM has a direct implication for intervention programmes as each of its components can be modified using traditional health education strategies; strategies for enhancing bednet usage that have been identified by an educator might be used in the HBM to reduce the perceived barriers to bednet usage.

The aim of this study was to use the HBM to predict malaria preventive behaviour.

**Materials and methods**
A structured questionnaire was developed to measure perceived susceptibility, seriousness, barriers to action, knowledge regarding the disease and its mode of transmission and cues to action. The validity of the items was tested during the pilot study. A cluster sample of 2168 housewives was randomly selected from the urban and rural areas of 5 districts (Kash, Saravan, Iranshahr, Nikshahr and Chabahar). Interviews were conducted by trained medical students.

**Major study findings**
The majority of the housewives (67.5%) and half of their husbands were illiterate. About one-third of subjects recognized malaria as an important disease but comparable to diarrhoeal illnesses, and only 50% identified mosquitoes as the vector responsible for disease transmission in spite of the high prevalence of malaria in the area. There was a relatively high frequency of misconception manifested by the belief that polluted water is the mode of transmission of the disease (21.3%). Such misconception has an adverse impact on preventive behaviour. There was a low frequency of bednet use (31%), mainly at night, while most of the mosquitoes' activity is at sunset. Less than half of the people attempted to dry water accumulations; it would therefore be beneficial to educate them in this context. The economic burden was the main reason for not screening the doors and windows in this area. The study indicated that adequate knowledge concerning the mode of transmission of the disease and the health benefits of bednet use resulted in an increase of bednet use as a preventive measure, while the perceived probability of acquiring malaria did not. By contrast, a considerable proportion of those who had adequate knowledge regarding the mode of transmission of the disease did not use bednets, indicating a need for a control programme to change public behaviour. However, the social context of malaria should be taken into consideration as the Western philosophy in educating individuals to take responsibility for their own health might not succeed in changing the individual's behaviour [4]. Therefore, a control programme could be more successfully achieved if implemented by the village headman or party officials than it can be by relying mainly on enhancing each individual's knowledge of the perceived risks.

**Recommendations**
Effective prevention programmes for malaria require the mobilization of community networks rather than education at the individual level. Community participation in the planning, implementation, monitoring and evaluation of a control programme is therefore recommended.

**References**
Investigation of sibling species of Anopheles fluviatilis complex in Iran

Islamic Republic of Iran
south-eastern provinces: Sistan and Baluchestan, Kerman and Hormosgan

Study period: 1999-2001

Small Grants Scheme (SCS) 1999 No. 56

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Abstract
This study aimed at applying different molecular techniques and cytogenetics to investigate the genetic variation between populations of the *Anopheles fluviatilis* complex belonging to different geographical areas of the south-eastern provinces with the highest burden of malaria in Iran. Mosquito sampling was performed during the seasonal activity of mosquitoes, and sibling species of the complex were identified. A modified protocol of Balinger-Crabtree yielded good DNA for PCR amplification. Eight RAPD primers were prepared, 3 were tested, and the RAPD-PCR reactions were carried out using 1 of these primers.

Results
Three populations of the *Anopheles fluviatilis* complex were identified in this region, and they belonged to Kazeron, Bandar-Abbas and Iran-Shahr. One hundred specimens were amplified for each population.

**UBC-306:** This primer reveals differences at the level of intra- and inter-population. At the level of intra-population it differentiated the 3 populations of Iran-Shahr, Bandar-Abbas and Kazeron.

**UBC-304:** This primer produced a series of bands for each population in which there was a single common band (690 bp) in all populations. As in the products of the UBC-306 primer, the products of the UBC-304 for Bandar-Abbas samples were mostly similar to the Kazeron samples. The last band (360 bp) was not detected in the Iran-Shahr samples. As this primer also detects intra-population variations, within the population of Kazeron, 2-3 distinct patterns could be identified.

**M13:** This primer produced a series of bands for each population in which there were two common bands (600 bp and 360 bp) in all populations.

Conclusion Molecular techniques identified 3 populations of the *Anopheles fluviatilis* complex in south-east Iran that belonged to different geographical areas. The UBC-306 primer reveals differences at the level of inter- and intra-population, whereas the M13 primer could not detect differences between populations or within the same population. This characterization of the species composition of the local population of a vector could be used in planning vector control programmes and controlling insecticide resistance.

Background
Malaria is the most important health problem in Iran, with up to 98 000 new cases diagnosed each year, with the majority (70%) occurring in the south-eastern (SE) provinces of Sistan-Baluchestan, Kerman, and Hormosgan. The *Anopheles fluviatilis* has been one of the incriminated vectors in this region. Distinct differences have been observed between the populations of this species in their feeding preferences, resting behaviour and infection rates. The identification of sibling species (S, T and U) was based on the banding pattern of polytene chromosomes, which is a cumbersome technique, time consuming, and necessitates skilled personnel and

Conclusions and implications of the study
- Molecular techniques identified 3 populations of the *Anopheles fluviatilis* complex belonging to different geographical areas of the south-eastern provinces of Iran. These genetic variants were from Kazeron, Bandar-Abbas and Iran-Shahr.
- Eight primers were prepared and 3 were tested: UBC-304, UBC-306, and M13.
- UBC-306 reveals differences at the level of inter- and intra-population. At the level of intra-population it differentiated the 3 populations of Iran-Shahr, Bandar-Abbas and Kazeron. UBC-304 could only detect intra-population variations, while M13 could not detect differences between populations or within the same population.
- Characterization of the species composition of a local population of a vector could be used in planning vector control programmes and controlling insecticide resistance.
a certain stage of development for the samples. Therefore, there is a need for an alternative rapid and sensitive method that could be applied on any stage of the mosquito. Previous studies on a limited sample using demonstrated DNA variations within populations of this species. This study aimed at applying different molecular techniques and cytogenetics to investigate the genetic variation between populations of the *An. fluviatilis* complex belonging to different geographical areas of south-east Iran.

**Materials and methods**

Mosquito sampling was performed during the seasonal activity of mosquitoes in the south-eastern provinces. Indoor and outdoor catching of adults was carried out and larvae were collected from breeding places. Identification of the sibling species of the complex was performed by examination of the banding pattern of polytene chromosomes present in the ovarian nurse cells of the adult. Reagents for the PCR and new primers (RAPD primers) were prepared in the laboratory.

**DNA extraction:** A modified protocol of Balinger-Crabtree [1] yielded a good DNA for PCR amplification.

**RAPD-PCR:** Eight RAPD primers were prepared and 3 were tested: UBC-304, UBC-306, and M13. Optimizing PCR reactions and troubleshooting were performed to produce good PCR products. The RAPD-PCR reactions were carried out using 1 of the previously mentioned primers.

**Main study findings**

Three populations of the *Anopheles fluviatilis* complex were identified in this region; they belonged to Kazeron, Bandar-Abbas and Iran-Shahr. One hundred specimens were amplified for each population.

The results of RAPD-PCR using each of the primers were as follows:

**UBC-306:** This primers reveals differences at the level of intra- and inter-population. At the level of intra-population, it differentiated the 3 populations of Iran-Shahr, Bandar-Abbas and Kazeron. Specimens of Iran-Shahr showed a sharp band at about 1115 bp, which was specific for this population. There were other bands which were not very clear, but among them there were 2 bands (at 400 and 370 bp) which divided the Iran-Shahr samples into 2 groups. Samples of Bandar-Abbas had 3 specific bands, at about 1320, 690 and 210 bp. At the level of intra-population, this primer revealed variations within this population. There were 2 different patterns within the population in which 1 group had a band at about 495 bp, whereas the other group had a band at about 470 bp. Most of Kazeron samples were similar to Iran-Shahr, however, there was a 495 bp band that was shared with Bandar-Abbas samples, and was not detected in those of Iran-Shahr.

**M13:** This primer produced a series of bands for each population in which there was a single common band (690 bp) in all populations. As with the products of UBC-306 primer, the products of UBC-304 for the Bandar-Abbas samples were mostly similar to the Kazeron samples. These 2 populations had a series of common bands such as the one at about 600 bp and another one at about 360 bp. This latter band (360 bp) was not detected in the Iran-shahr samples. As this primer also detects intra-population variations, within the population of Kazeron, 2-3 distinct patterns could be identified.

**UBC-304:** This primer produced a series of bands for each population in which there were two common bands, 600 bp and 360 bp, in all populations. The results of this study could be used in a malaria control programme to gain a better understanding of vector transmission and disease epidemiology, and to optimise vector control programmes.

**References**

Malaria Morbidity and Mortality

Somalia

Abstract

This study was undertaken to identify the determinants of severe malaria morbidity and predictors of mortality from malaria. All cases admitted to Hargeisa, Berbera and Borama hospitals were included in the study. Complicated cases accounted for more than one-third of admitted cases. Diagnostic signs and symptoms of severe malaria were: vomiting, high temperature, anaemia, dehydration, dizziness progressing to coma, oliguria, proteinuria, parasitaemia, icterus, dry cough and epistaxis. Determinants of complications in malaria cases included: infection with Plasmodium falciparum, diagnostic and treatment delay, wrong health beliefs regarding the disease, suboptimal quality of care, drug resistance, and co-morbid conditions (chronic renal disease, tuberculosis, chronic hepatitis, malnutrition and parasitic infestations). A negative past history of malaria was strongly associated with encountering a severe malaria attack. Predictors of mortality from severe malaria were cerebral and renal involvement.

Background

Malaria outbreaks and epidemics in Somaliland began in 1977 with the flow of refugees from Ethiopia following the war between the two countries. The deterioration of the health infrastructure during the post-war period interfered with the achievement of a successful malaria control programme in the country. Accordingly, the case fatality rate from malaria was increasing, though underestimated due to the lack of a reliable registry system. Plasmodium falciparum is the predominant protozoa and is mostly transmitted by the vector Anopheles arabiensis.

This study was undertaken to identify the determinants of severe malaria morbidity and predictors of malaria mortality in the disadvantaged conditions of one of the least developing countries.

Activities achieved within the framework of the study

It was a great accomplishment for Somaliland to carry out such a study while other regions in Somalia were under an emergency situation and categorized as war zone. The research faced many constraints attributed mainly to the deteriorated health infrastructure and low health staff motivation.

Materials and methods

All cases admitted to Hargeisa, Berbera and Borama hospitals were included in the study. The items on the questionnaire were assessed for their validity. The questionnaire included information regarding: demographic data, past history of malaria, reason for admission, details of clinical examination and laboratory investigations, as well as medications given before and after admission. The field investigators were asked to write the details of follow-up and the outcome of treatment.

Case management

Management of severe malaria cases was generally according to the recommended WHO guidelines. Treatment approach to the patients depended upon the general condition of the cases. Rapid clinical assessment was first carried out to evaluate the overall condition of the patient then drug prescription was performed. If the patient is comatose or semicomatose, Quinine Di-hydrochloride
injection was the first drug of choice. In case of dehydration (vomiting, diarrhoea, high fever) ringer lactate was given, and blood transfusion was given in case of anaemia (Hb<6mg/dl). Palliative drugs such as antipyretics, and analgesics were also given whenever needed.

Main study findings

**Hargeisa General Hospital** Over 9 months, 312 total malaria patients were admitted, with severe complicated cases constituting 38.1% of cases (119 patients). The case fatality rate was underestimated (3.3%) as most of the patients were managed in the private sector. Two-thirds of the admitted patients were male. This is attributed to the high occupational exposure of males to the vector in agricultural fields, but might also reflect gender-related inequalities in accessibility to health services.

**Berbera General Hospital** The study was interrupted 3 months after its onset due to the overall decline in the number of admitted patients because of unfavourable climatic conditions. However, during this shortened period, 12 out of 60 admitted malaria cases were complicated (20.0%), and no deaths were recorded.

**Borama General Hospital** Over 7 months (July 1996-January 1997), 125 patients with severe malaria were admitted, with the highest percentage in October, and the lowest in January. Severe cases constituted 34.3% of all malaria patients (125/364 admitted), with a case fatality rate of 10% of all admitted patients and 29% of severe cases.

**Diagnostic criteria of complicated malaria cases** Complicated cases accounted for more than one-third of all admitted malaria patients in the 3 hospitals (256/736 admitted). The following signs and symptoms were diagnostic of complicated malaria: vomiting, high temperature, anaemia, dehydration, cerebral dizziness to coma, oliguria, proteinuria, parasitaemia, icterus, dry cough and epistaxis.

**Determinants of complications in malaria cases** The severity of malaria resulted from an interplay of factors which included: infection with *P. falciparum*, diagnostic and treatment delay, wrong health beliefs about the disease, suboptimal quality of care, drug resistance, and co-morbid conditions (chronic renal disease, tuberculosis, chronic hepatitis, malnutrition and parasitic infestations). A negative past history of malaria was strongly associated with encountering a severe attack of malaria (60% of severe cases). Predictors of mortality from severe malaria were cerebral and renal involvement.

**Diagnostic and treatment delay** Delay in diagnosis and treatment appeared to be a significant determinant of severe malaria as more than 80% of cases were referred from remote areas to the studied hospitals to receive appropriate care. Reasons for diagnostic and treatment delay were: living at a far distance from the health facility (>50 km); lack/difficulty in transportation; rough roads; or due to the use of non-prescribed medications at home. In other instances, patients sought care late due to superstitious beliefs regarding the causes of malaria such as: chronic constipation, toxins injected by mosquitoes, evil or Satan, and drinking water contaminated by the eggs of mosquitoes.

**Evaluation of malaria management** On some occasions, physicians were not strictly adherent to the WHO guidelines in patient management. Drug resistance to chloroquine and sulfadoxine/pyrimethamine was recorded.

**Recommendations**

The information gathered in this study highlights the need for educating health professionals on the recent therapeutic guidelines and the community regarding the disease, ensuring the availability of malaria medications in health facilities, and improving patients' access to health services.
Abstract
This study was carried out to evaluate the effect of community use of bednets impregnated with insecticide (permethrin) on the control of the malaria vectors in Sudan. Two villages, Alakafek and Eredeba, were selected for the study. Thick blood films stained with Giemsa stain were obtained from a randomly selected sample of 160 villagers from each village. The parasite density was recorded and all positive patients were weighed and treated with chloroquine (25 mg/kg body weight). Individuals were also interviewed regarding malaria symptoms. Indoor and outdoor collection of mosquitoes from 7 randomly selected houses was carried out. The community and health authorities were educated regarding impregnated bednet usage. The efficacy of the insecticide-impregnated bednets was evaluated on the mosquitoes collected from the study and control villages.

Results
Anopheles pharoensis was the predominant species of mosquitoes collected in the study area, followed by An. arabiensis, with the highest frequency in December. Bioassay results indicated significantly higher mortality rates in the experimental group exposed to insecticide-impregnated bednets compared to the control group. The prevalence of positive blood tests during the baseline survey was 12%, which was significantly reduced to 2.5% after intervention. There was a significant difference between both villages regarding the overall prevalence of positive cases. The prevalence of malaria infection was highest among individuals aged 20-29 years and the protective effect of insecticide-impregnated bednets was more evident among children up to 10 years old. Malaria symptoms were present in 55.6% of cases and 60% of controls, and the maximum parasite density was less in the study group.

Conclusion
The results of this study showed that insecticide-impregnated bednets are an efficacious method for vector control in endemic regions.

Background
Malaria is considered one of the major public health problems in Sudan, with profound impact on the productivity of individuals. As farmers are the most vulnerable group, a widespread malaria infection among farmers during the rainy season directly affects agriculture, the country’s main resource. Insecticides for use in vector control have been generally applied throughout the country, resulting in HCH and DDT resistance. The use of biological vector control, such as larvivorous fish, was not previously practised, and the use of bednets was very limited and often incorrect. This study was carried out to evaluate the effect of community use of bednets impregnated with insecticide (permethrin) on the control of malaria vectors in Sudan.

Materials and methods
Study area Two villages, Alakafek and Eredeba, were selected for the study. Alakafek village was selected for intervention, while Eredeba was taken as the control. The breeding sites were in low land that retains excess flood and seepage from canals. The population consisted of farmers living in huts made of thatch and in small mud dwellings.
Baseline survey (January-May 1996) Clinical and parasitological survey: Thick blood films stained with Giemsa stain were obtained from a randomly selected sample of 160 villagers from each village. The parasite density was recorded per 100 fields of high power. All positive cases were weighed and treated with chloroquine (25 mg/kg body weight). Individuals were also interviewed regarding malaria symptoms.

Vector survey: Mosquitoes were collected at 6:00 and 18:00. Seven houses were randomly selected for space spraying and knock-down collection. The parity rate was determined by records of the ovarian tracheoles, and salivary glands were crushed for sporozoites.

Intervention phase (June 1996-July 1997) The community and health authorities in Alakafek village were educated regarding impregnated bednet usage. Bednets made of opaque cotton fabric, primarily used to protect against sandflies, were impregnated over 2 weeks to give a uniform 200 mg/m insecticide coating.

Bioassay of the efficacy of insecticide-impregnated bednets The efficacy of the insecticide-impregnated bed nets (IIBN) was evaluated on 3-to-5-day-old adult mosquitoes collected from the study and control villages. The tests applied were in accordance with the method described by Sohreck, using the WHO plastic cones. Seven tests were performed to test the IIBN's efficacy: 3 tests after the first impregnation, 3 tests after the second impregnation, and the last test after reimpregnation and replacement of lost nets.

Main study findings

_A. pharoensis_ was the predominant species of mosquitoes collected in the study village (80% and 88% of indoor and outdoor catch, respectively), followed by _A. arabiensis_. The highest frequency of indoor or outdoor collected mosquitoes was recorded in December for both species in the study village and for _A. pharoensis_ in the control village. A significantly lower percentage of _A. arabiensis_ was recorded in the control village compared to the study village.

The prevalence of positive blood tests during the baseline survey in Alakafek village was 12%, mainly _P. falciparum_ (11.6%), and rarely _P. malariae_ (0.4%). The intervention phase resulted in a significant reduction in the frequency of positive malaria cases to 2.5%. Furthermore, there was a significant difference between both villages regarding the overall prevalence of positive cases (2.5% in the study village compared to 8.1% in the control village). The prevalence of malaria infection was highest among individuals aged 20-29 years. It is noteworthy that the impact of impregnated bednets was more evident among younger age groups; no children under the age of 5 were diagnosed, and only 0.6% of children 5-9 years old were positive for malaria in the study village, compared to 8.4% and 7.1% for these age groups, respectively, in the control village. The frequency of positive cases was slightly higher in females (M:F ratio of 1:1.45 and 1:1.14 in the study and control village, respectively). Malaria symptoms were present in 55.6% of cases and in 60% of controls, while the maximum parasite density was 635 in cases compared to 1630 in controls. Similarly, bioassay results indicated significantly higher mortality rates of adult mosquitoes in the experimental group exposed to IIBN compared to the control group.

The results of this study showed that insecticide-impregnated bednets is an efficacious method for vector control. It is recommended that impregnated bednets be used to achieve high control resolution of malaria vectors in highly endemic regions throughout the world. This study also indicates the beneficial effects of educating communities, regardless of their educational levels. The role of health education in malaria vector control is therefore emphasized.
Abstract

This study was undertaken to evaluate the impact of several parameters of hill lakes on the density of the larval population and how these may affect the biological control of the larval population using natural predators. The prospective study was conducted over a 1-year period in north-western Tunisia, where 6 lakes were randomly selected and paired. Within each pair, one lake was chosen as a study lake and the other as a control. The physicochemical properties of the water were studied in the field as well as in the laboratory. Sampling of larvae and vegetation was performed twice monthly, and specimens were transported to laboratories for further studies. Random sampling of Gambusia affinis holbrooki from Lebna Dam was performed twice monthly, and specimens were transported to laboratories for further studies. Specific numbers of larvae were introduced into an aquarium and the efficacy of predation was determined.

Results

The physicochemical analyses of the water revealed that water quality in the hill lakes varied considerably and that they were suitable breeding localities for Anopheles labranchiae, Culex theileri, Culex pipiens, Aedes caspius and Culiseta longeriolatta. The frequency of larvae varied in the different lakes, with C. theileri as the most frequent and constant species in all types of lakes. The introduction of Gambusia affinis fish in the 3 studied lakes resulted in a significant decrease in larval population compared to the control group.

Conclusion

The efficacy of predation was related to the size of the larvivorous fish and the water temperature while negatively influenced by the presence of vegetation. It is therefore recommended to introduce herbivorous fish together with larvivorous fish to achieve successful biological vector control.

Background

A national malaria control programme was implemented in Tunisia in 1968, resulting in the disease being completely eradicated. Since then, health authorities have followed a preventive policy against malaria by controlling the factors favouring the reintroduction of the disease. The lakes that were sited at the foot of hills throughout the country, primarily as part of irrigation projects, became favourable breeding sites for the vectors of the disease. Vector control therefore emerged as a public health concern that needed to be tackled while respecting the environment. In fact, the biological control of mosquitoes has always been considered the most appropriate method for vector control. It includes the use of vertebrate and invertebrate predators and enteropathogens such as viruses, bacteria (Bacillus sphaericus and B. thuringiensis), fungi, protozoa, nematodes, and larvivorous fish.
Gambusia affinis hollbrooki fish is considered an efficient natural predator of anopheline larvae and was introduced in Tunisia in 1929 in the La Mannouba artificial breeding basin. This study was undertaken to evaluate the impact of several parameters of hill lakes on the density of larval population and how these may affect the biological control of the larval population using natural predators.

Materials and methods

The prospective study was conducted in northwestern Tunisia (Beja and Bizerte governorates). Six representative lakes were randomly selected, and matched for similarities between them and the timing of their construction, thereby forming 3 matched pairs of lakes: recent (1994), intermediate (1990-1992), and old (1984-1987). This subdivision was chosen mainly to study the effect of the development of vegetation with time as a factor favouring the breeding of the vector. Within each pair, one lake was chosen as a study lake and the other as a control.

The physicochemical properties of the water were studied in the field as well as in the laboratory. The field survey consisted of larval sampling by the dipping method whereby a container of known capacity was plunged at randomly selected points in the lake and the number of larvae per dip was determined. Knowing the volume of water in the lake, the total number of larval population was estimated using a special formula. The collected larvae were then transferred to the laboratories for further studies. The field survey also included the random sampling of vegetation, and these samples were transported to the laboratories for analysis.

Laboratory analyses The larvae were separated according to their developmental stage. Larval counting was performed on mature larvae (stage IV) using binocular lenses. Breeding of immature larvae and nymphs took place in suitable conditions, till stage IV (for larvae) or adult stage (for nymphs) was reached.

Larvae and adults were then identified and preserved in a suitable container. The relative and seasonal frequencies of species and several indices providing information regarding the predominance of different species in different stations were calculated, and the impact of modification in the biotope on the larval population was measured.

Biological control Gambusia fish were released into the study lakes (3300 in Sidi M’barek, 3160 in Ain Elbabouch, and 1220 in Skhira). Another sample of fish was transported to the laboratory, kept in an aquarium, and fed on artificial material. A defined number of larvae was then introduced into the aquarium and the number of larvae consumed by the fish was determined. The effects of vegetation, larval movement and water temperature on the predators’ efficacy were recorded.

Main study findings

The physicochemical analyses of the water revealed that the water in the hill lakes varied from sweet to low salinity, from moderate to strong alkalinity, and all were well oxygenated. Nitrogenous compounds varied according to the month of sampling, and the waters were rich in organic compounds. These lakes were suitable breeding localities for mosquitoes, namely: Anopheles labranchiae, Culex theileri, Culex pipiens, Aedes caspius and Culiseta longirostrata. There were also the most frequent and invariable species in all types of lakes. On the other hand, An. labranchiae was absent from O. Elgraah lake due to its turbidity and water pollution. However, this type of water is more favourable for the breeding of C. pipiens and C. longirostrata. A. caspius was rare due to the low salinity of these lakes. The introduction of Gambusia affinis fish in the 3 studied lakes resulted in a significant decrease in the larval population compared to the control group.

Laboratory studies also indicated a significant association between the size of larvivorous fish and the number of larvae consumed, a lack of preference for any particular species, although the fish prefer live rather than dead larvae, and that the efficacy of predation was higher with increasing water temperature. The diversity of mosquito species was mainly dependent on the heterogeneity of the environment; Lake O. Elboul, the richest in vegetation, recorded the highest diversity of species. The presence of vegetation was the main determinant of high larval density. This rich vegetation provides organic substances favouring the growth of the microorganisms upon which the larvae feed. This vegetation constitutes a habitat for these larvae, and when dense, it is an obstacle that interferes with the predating efficacy of the Gambusia fish. It is therefore recommended that herbivorous fish such as Ctenopharyngodon idella or Cyprinus carpio be introduced along with the larvivorous fish. Biological control of mosquitoes using Gambusia affinis and Cyprinus carpio, the latter also being a protein-rich food source for humans, could be an environmentally safe and cost-effective alternative to pesticides for achieving successful vector control.
Abstract
An intervention study conducted in Al Rahad area aimed at an assessment of the effectiveness of impregnating thobs with insecticide as a vector control method. Two villages were selected as study and control villages.

Results
The prevalence of malaria decreased significantly in the study village 1 year after intervention (from 53.8% to 3.5%) and over the 4 follow-up surveys. A high protective efficacy of 81% was attributed to the impregnated thobs. All households were compliant to the use of the impregnated thobs, and kept them folded in their houses. There was a high acceptability of this method by the villagers and negligible side effects were recorded. The thobs had a significant impact on the mortality rates of the mosquitoes. Anopheles arabiensis and An. rufipes were the 2 female anopheline mosquitoes identified in the 2 villages.

Conclusion
Insecticide-impregnated thobs are an effective and safe vector control method in endemic areas, with high acceptability from the community.

Activities conducted in the framework of the project
The community health workers in the village health centre, as well as school teachers, were motivated regarding educating the community and students concerning malaria control.

Background
The thob is a wrap, normally made of cotton, worn by women in Sudan over their regular dresses. It is also used as a cover for women and their children while sleeping. In view of this habit, it was hypothesized that these thobs, once impregnated with insecticides, would be a more acceptable and cost-effective vector control method compared to impregnated bednets, particularly since village huts are too small to accommodate bednets. The assessment of the effectiveness of impregnated thobs as a vector control method and the education of the community on the impregnation method were the objectives of this study.

Materials and methods
Two endemic villages in the Al-Rahad area were selected: Elregeila and Tendalti as intervention and control villages, respectively.

Parasitological survey
Thin and thick blood films were examined by Giemsa stain. A pre-intervention survey was conducted on a sample of 338 and 213 villagers from the study and control villages, respectively, followed by 4 surveys, 2 during and after the transmission season. Febrile villagers in the study village were also parasitologically examined, and positive cases treated.

Thobs distribution and treatment
The thobs were distributed to all 600 villagers in the study village. Each household was visited and the names of the thobs users were recorded. The thobs were made from local cotton fabric (wilaya). The insecticide used in treating the thobs was Deltamethrin (K-Othrin EC 2.5). A big tree in the centre of the village...
was selected as the station for the impregnation. The
new thobs were rinsed first in a measured amount
of water and soaked in a bucket until totally wet.
The required amount of insecticide for each thob was
calculated. The thobs were then treated by dipping
them in a mixture of insecticide and water. Each thob
was soaked until the insecticide was completely
absorbed and then laid flat to dry in the shade.

Bioassay and effectiveness of insecticide-
treated thobs The effect of 1, 2, and 3 washes of
the treated thobs was tested. Adult mosquitoes were
exposed to thob pieces (impregnated, control and
washed) for 3 minutes and a piece of cotton wool
soaked with glucose solution was provided at the
end of a holding tube on the nylon gauze during the
24-hour holding period. Mortality was recorded 24
hours after exposure.

Impregnated thobs evaluation
questionnaire An evaluation questionnaire was
designed to assess the acceptance of the community
regarding the thobs and side-effects were recorded.

Night bite collection (NBC) Night bite collection
was performed monthly in sentinel sites in both
villages, whereby human-bait collectors performed
the catches in 2 shifts between 18:00 and 06:00. The
catchers were given chloroquine tablets for
chemoprophylaxis. Mosquitoes were collected while
landing on the host to bite, therefore, the collectors
themselves acted as bait. A torch was switched on
when a bite was felt and the mosquitoes were sucked
into an aspirator. The mosquitoes were then
transferred into paper monocups. Anopheline
mosquitoes were identified morphologically by the
key method, counted and dissected for sporozoites.

Pyrethrum Space Spraying Collection (PSC)
PSC was performed on the morning following night
bite collection in a randomly selected room in each
of the 5 clusters dividing each of the 2 villages. The
selected room was considered a sentinel site. After
spraying, the mosquitoes were collected and placed
in Petri dishes on moist filter-paper (Whatman no.
1). Anopheline mosquitoes were identified and
counted, females were classified by stages of
abdominal appearance and then dissected for sporozoites.

Main study findings
There was a significant difference between the
study and control villages regarding the overall
prevalence of malaria (41.1% and 18.8%,
respectively). The prevalence of malaria decreased
significantly in the study village 1 year after intervention
(from 53.8% to 3.5%). Furthermore, there was a
significant difference between the study and control
villages regarding the prevalence over the 4 follow-
up surveys. A high protective efficacy of 81% attributed
to impregnated thobs against malaria infection was
obtained during the transmission season in the study
village.

The thobs were re-impregnated twice and all
remained in a good condition during the study period.
The cost of the impregnated thob per person was LS
3650.

The KAP study carried out at the beginning of the
study indicated that the type of houses in the study
village is huts and most people sleep outdoors, the
villagers knew about malaria as a disease, but were
not aware regarding its transmission or control
measures. No previous insecticide spraying had been
performed in the village or its surroundings.

All households were compliant to health education
sessions and to the use of impregnated thobs, and
kept them folded in their houses. The evaluation
showed the high acceptability of the method by the
villagers in terms of material quality, adequateness,
affordability, and negligible side effects (1.5%). They
used it for all family members, indoor and outdoor.

The bioassay test showed that 100% knock down
ocurred within 3 minutes of exposure of An.
arabiensis to the treated thob. All the knock-down
mosquitoes died after a holding period of 24 hours.
The thobs washed for the first and second time gave
100% knock down within 3 minutes and 100% mortality.
The bioassay on thobs used for 3 months showed
97.5% mortality after 24 hours and 3 minutes' exposure.
For thobs washed once and twice, the
mortality was 83.3% and 33.3%, respectively. The
bioassay on thobs used for 11 months showed 93.3%
mortality after 24 hours and 3 minutes' exposure, and
40% and 26.6% mortality rates for the first and
second washes, respectively.

Two species of female anopheline mosquitoes
were identified, An. arabiensis and An. rufipes, in the
2 villages. There was seasonal fluctuation in the An.
arabiensis density in both villages, appearing only in
the wet season (July to October). The indoor resting
density was very low, and there was no significant
difference between both villages regarding the
mosquito resting density. This species was found to
be strongly endophagic as about 60% were captured
while trying to bite human baits indoors. The earliest
period of activity was found at 18:00, indoor and
door. Biting continued overnight with a marked
increase of the biting period, both indoor and outdoor,
occurring between 20:00 and 02:00, with a peak at
22:00-00:00, then gradually declining indoors at
02:00-06:00 while disappearing outdoors.

Regarding human-biting rates, An. arabiensis biting
activity reached its peak in October. An average
villager was bitten by 2.7 and 3.4 females nightly in
the study and control villages, respectively, during
the wet season (October-January), and no bites were
recorded in the dry season.
Comparative efficacy studies using alternative pesticides (biopesticides, insect growth regulator and ivermectin) on anopheline-malaria mosquitoes in Egypt

**Egypt**
Fayoum, south-west of Cairo

**Study period:**
December 1998–November 1999

**Small Grants Scheme (SGS) 1998 No. 47**

**Principal Investigator**
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**Abstract**
The aim of this study was to assess the effectiveness and safety of different pesticides for use in areas at risk of malaria. A larval survey was carried out in Fayoum, Egypt to determine the larval density. Water and sediment analyses were performed on samples from mosquito breeding sites. The potency of different pesticides, including biopesticides, insect growth regulators and ivermectin, and their mixtures, on larvae was assessed in the laboratory as well as in the field. Their efficacy was expressed as the mean percentage reduction of larvae in treated versus control pools.

**Results**
All the pesticides tested exhibited significant larval reduction of the malaria vector. Recently introduced ivermectin, triflumuron and neem (alone or in combinations) proved to be equally efficacious and long-lasting.

**Conclusion**
The study emphasized a combined water management and neem coating strategy as an environmentally safe method that could be implemented as a control measure for rice-field mosquitoes. Neem was recommended as a measure for achieving high control of the malaria vector in endemic regions.

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**Conclusions and implications of the study**
- All pesticides tested exhibited significant larval reduction of the malaria vector with long-lasting effect (up to 30 days post-treatment).
- A combined water management and neem coating strategy could be implemented as a control measure for rice-field mosquitoes (*Anopheles pharoensis*), to minimize the hazards of possible malarial transmission in the future.
- The high frequency of the malaria vector *A. sergenti* could be attributed to the hydraulic situation of subsoil water, hindering the spraying of swamps with weeds and wild plants, and the suitable meteorological conditions. Agricultural and other development projects and land excavation have resulted in development of new breeding sites, which may lead to increased transmission in the future. The utmost need for vector control measures with the studied pesticides, even in low-risk areas, is emphasized.

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**Background**
Fayoum Governorate is considered the sole focus of malaria in Egypt. *P. falciparum* is the predominant species recorded, since *P. vivax* was eradicated in 1996. The aim of the study was to evaluate the efficacy of new and frequently used alternative pesticides such as *Bacillus thuringiensis israelensis* and *Bacillus sphaericus* formulations under laboratory and field conditions, as a means for developing an efficacious and integrated vector control method.

**Materials and methods**

**Study area**
Fayoum is a large agricultural oasis at an altitude of 20 m below sea level. Selection of the study areas in Sinnuris and Fayoum districts was based on the frequency of malaria cases and the proximity of breeding sites to residential areas. The permanent stagnant water sites were randomly selected to represent all parts in each district. Information pertaining to the land characteristics and breeding places was recorded.

**Monthly larval survey**
A monthly larval survey was carried out, and sites with high larval density were chosen as fixed catching stations. Larvae were collected using the dipping method [1] and transported to the laboratory to be counted and identified. Ten dips were
taken from each location, and the relative density of mosquitoes as a larval index (number of larvae/dip) was calculated monthly.

**Rice-land survey (July-October 1999)**
Sampling along the rice fields was carried out using the standard dipper. Collected anopheline larvae were counted and identified.

**Laboratory tests:**

**Water samples** Water samples were randomly collected from different locations in the breeding sites and were categorized according to larval intensity (high, moderate or negative). The chemical and physical properties of the water, and the presence of pesticide residues were determined [2].

**Sediment samples** Sediment samples were collected from the upper layer of the pool bottom and analysed.

**Laboratory experiments** Laboratory experiments included the assessment of the potency of different alternative pesticides and their mixtures on the larvae.

**Field trials** Field trials were conducted to assess the optimum effective doses of the pesticides in the field, whereby pre-weighed amounts of different formulations and their mixtures were sprayed onto the surface of ponds as treatment measures. They were then evaluated for their efficacy as the mean percentage reduction of larvae in treated versus control pools.

Standard statistical tests were performed. Bioassays were analysed using probit analysis that computes the LC$_{50}$ and LC$_{95}$ and chi-square goodness of fit for comparison.

**Main study findings**
*A. sergenti* was the most frequent species (79%), followed by *A. multicolor* (20.8%), of the collected larvae. All tested alternative pesticides exhibited significant larval reduction of the malaria vector (*A. sergenti*) with long-lasting efficacy (up to 30 days post treatment).

There was no significant difference in the percentage larva reduction between ivermectin, *Bacillus thuringiensis* (VectoBac 12AS, VectoBac G), lufenuron (Match) and neem 24 hours post treatment, while *Bacillus sphaericus* (ABG-6490) was the least effective. The corncob formulation (VectoBac G) proved to be more efficacious and long-lasting than the fluid concentrate (VectoBac 12AS). Recently introduced ivermectin, triflumuron and neem (alone or in combinations) proved to be equally efficacious and long-lasting.

The combinations used in this study successfully reduced the larval population density to less than 1.00 larvae/dip (91%-100% larval reduction) up to 30 days post treatment, with LC$_{25}$ TFM-LC$_{25}$ DLM combinations as the most efficacious of all.

A combined water management and neem-coating strategy is an environmentally safe method and was acceptable to farmers due to the increase in the yield of cultivated crops. Therefore, it could be implemented as a control measure for rice-field mosquitoes (*A. pharoensis*) to minimize the hazards of possible malarial transmission in the future.

Furthermore, owing to its protective effect against adult mosquito biting, it is recommended to impregnate bednets and clothes with neem in order to achieve high control resolution of the malaria vector in highly endemic regions throughout the world.

This study finally suggested that an information system built on the malaria vector control programme could have a strong impact on communities, incorporating health education, water provision, sanitation and the establishment of primary health care.

**References**
Evolving a social marketing approach for promoting the use of insecticide-impregnated bednets in rural Islamabad

Pakistan
rural Islamabad

Study period:
November 1998–August 2001

Small Grants Scheme (SGS) 1998 No. 65

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Abstract
This project was designed to develop feasible and acceptable approach(es) for the social marketing of insecticide treated nets (ITNs) in a rural population, through primary health care outlets. Two villages in rural Islamabad were subjected to context analysis. The social marketing strategy was evolved in a series of meetings with various stakeholders. The primary health care outlets and active support of community-based health workers and community-based organizations in the project area were used to promote, distribute and provide health education about the ITNs. A 3-phase approach was adopted: A total of 110 bednets were sold during 6 months. Data from buyers and non-buyers were collected.

Results
In order to make the price of ITNs affordable to the community, shifting the strategy from a single payment to installments, the use of double-bed size ITNs, and minimizing the difference in the price between insecticide-impregnated and non-impregnated bednets, were the solutions devised. Vaccinators and female health workers were the most common source of information and purchase of ITNs. An easy mode of payment was considered the most frequent enabling factor in purchasing bednets, followed by the quality of the bednets and malaria prevention. Almost all users paid installments on time.

Almost two-thirds of non-buyers knew about the availability of ITNs. The main factors hindering non-buyers from purchasing ITNs were the lack of conviction about the utility of ITNs, high cost, and use of other methods. Their main suggestion for promoting ITNs was increasing community awareness.

Conclusion
The experience of marketing ITNs through public-private partnership was found to be very useful.

Background
The main emphasis of malaria control programme nowadays in Pakistan is on sustainable control programmes that can be implemented in the context of primary health care systems through active community participation. This project was designed to develop potentially feasible and acceptable approach(es) for the social marketing of impregnated bednets (ITNs) in a rural population through primary health care outlets.

Materials and methods
Two villages in rural Islamabad with a total population of 17,250 were selected. Context analysis was conducted by reviewing the literature/records, interviewing key informants and conducting community discussions. The data collection methods included the village profile tool, community dialogue and care-provider dialogue forms. The social marketing strategy was evolved in a series of meetings, interactions, and discussions with various stakeholders, using a separately designed tool, i.e. a strategy evolution guideline.

The primary health care outlets and the active support of community-based health workers, i.e. vaccinators and female health workers in the project area, were used for promotion, distribution and health education of the ITNs. Also, the support of community-based organizations in the project area was utilized to facilitate the performance of ITN-related activities by
primary health care outlets by highlighting specific roles and responsibilities. The bednets were made available for purchase in a single payment or installments on a not-for-profit basis.

A 3-phase approach was adopted to operationalize/pilot the marketing strategy. In the initial strengthening phase, the baseline supply of 10-15 ITNs and information materials was supplied in advance to the selected primary health care outlets, health workers and members of community-based organizations. Training on the proper technique of managing ITNs and their installation was given to the concerned individuals. The second phase consisted of establishing protocols of the detailed mechanisms for procurement, initial investment, baseline supply, selling, cash management, record keeping/follow-up, promotion, health education and promotional materials, training and sustainability. The last phase consisted of supervising and monitoring the implementation process by monthly visits to the health facilities, health workers and members of the community-based organizations. To keep an updated information system for the management of cash/installment payments, a customer profile and a monitoring system for the installment tool was developed. An extensive exercise was designed and conducted to assess the evolved strategy. A multi-method approach was followed including qualitative and quantitative data collection and analysis to understand the providers’ and consumers’ perspectives of the ITN experience. Focus group discussions were held to share and learn from the experience of providers involved in implementing the social marketing strategy. Data from buyers and non-buyers were collected by using a structured questionnaire. A total of 110 bednets were sold during 6 months. A total of 56 households who had bought 1 or more of these ITNs were interviewed. Information was collected on their socio-demographic background and on their knowledge, attitudes and practices concerning bednets. For comparison purposes, this same information was also collected from 56 non-buyer households, from the house to the right of each buyer house.

Main study findings

The price of the bednets was not affordable for many of the community members. Shifting the strategy from a single payment to installments was found to be effective. Female vaccinators and health workers showed lower interest compared to males, probably due to the fact that the difference between the price of impregnated and non-impregnated bednets needed to be minimized. There was no significant difference between buyers and non-buyers regarding socio-demographic variables, their awareness that mosquitoes are a nuisance and/or a health risk, or their history of normal bednet usage. The current buying practices were not influenced by past history of use. The majority of individuals claimed that they had used some sort of protective measure against the mosquito bite, mainly fans. There was no significant difference between the 2 groups regarding the cost of protective measures used prior to their introduction to ITNs, the difference in the size of catchment population, and female clients having relatively limited decision-making regarding ITNs use. It was found that it was easier to demonstrate the use of ITNs to groups compared to a single buyer. The use of double-bed sized ITNs was found to be more economic and acceptable. Regarding the different sources of information about ITNs, vaccinators and female health workers (64.3%) were the most common source of information, followed by members of community-based organizations (19.6%), then health facilities posters/pamphlets distributed in the area (each 5.4%). Community-based health workers, including vaccinators and Lady Health Workers, and members of community-based organizations were the main source of ITNs. The easy mode of payment was considered the most frequent (46.4%) enabling factor in purchasing bednets, followed by the quality of the bednets and malaria prevention.

The major reported reasons for non-use were that ITNs were purchased for someone else or that it was late in the mosquito season. Among the users, 77.5% used the bednets while sleeping outdoors, 10% used them only indoors, and 12.5% used the bednets both indoors and outdoors. The majority said that there was no constraint to the use of bednets, while others said that installation was a cumbersome process, but no one mentioned hot, windy, or humid weather as a constraint. Almost all users were convinced about the efficacy of ITNs and recommended them to their relatives. They also paid installments on time.

Almost two-thirds of non-buyers were informed about the availability of ITNs through community-based health workers, members of community-based organizations and public meetings, but rarely through health facilities or general neighbourhood talk. Purchasing of the ITNs was also through the same sources.

The main factors hindering non-buyers from purchasing ITNs in spite of their knowledge about their availability were: lack of conviction about the utility of ITNs, high cost, and use of other methods. Their main suggestions for promoting ITNs were: increasing community awareness through interpersonal communication, followed by mass announcement through mosques, distributing posters/pamphlets to homes, organizing community meetings and holding public demonstrations.
Abstract
This study was conducted in Fayoum Governorate to determine the laboratory and field efficacy of neem oil, Temephos and Chlorosol towards anopheline larval density in different water bodies, and to study the preliminary toxicological impact of neem oil on non-target species. FEBA® detergent was used to emulsify the neem oil. A village was divided into 4 sectors. The water bodies in each sector were then treated with Temephos, Chlorosol, or neem oil, and 1 sector was left as a control. To study the field efficacy of neem oil as an alternative larvicide, all water bodies in one village were treated with neem oil while those of another village were treated with Chlorosol. Treatment was applied biweekly for 7 continuous rounds in the summer. Larval density was monitored for the baseline and regularly thereafter until the 4th week of post-treatment.

Results
Neem oil and other tested compounds showed variable degrees of toxicity for the non-target species. The safety of the neem oil was also proved by testing it on laboratory mice. Water bodies were free of anopheline larvae for 4 weeks after a single application of Temephos or Chlorosol compared to 2 weeks in neem oil-treated water bodies. No anopheline larvae were detected after 1 week of applying the tested larvicides. Moreover, after two weeks. The larval density was dramatically reduced with no significant difference in the anopheline larval density between the 2 treated villages. Residual spraying of neem oil on the walls and ceilings of the dwellings also showed a significant reduction in the number of anopheline adults and their positive sites.

Conclusion
Neem oil is not recommended as an alternative larvicide unless justified by acute insecticide resistance.

Background
Egypt has been freed from malaria, except for 2 districts in Fayoum Governorate; Sinnuris and Fayoum. However, there is always the risk of resumed transmission due to the presence of the anopheline species in this area and the long transmission season of Plasmodium falciparum. This study aimed at evaluating neem as an alternative larvicide, and comparing it to other larvicides in the prevention of resumed transmission in this area. The neem tree (Azadirachta indica) is a tropical evergreen. Azadirachtin, one of the first active ingredients isolated from neem, has proven to be a primary agent for vector control. The simplest neem pesticide is a crude extract, however, advanced formulations possess antifeedant, repellent, ovipositional inhibitor and insecticidal properties.

Materials and methods
Two villages in Sinnuris District, Fayoum Governorate, were selected for implementation of this study: El-Henawy and Abheit El-Hagar villages.

Conclusions and implications of the study
The efficacy of azadirachtin as an insecticide is well documented in the literature. However, the results of this study cannot support large-scale use of neem oil unless justified by acute insecticide resistance problems. Neem oil is not selective for resistance since azadirachtin is a natural mixture of natural substances with various modes of action.

It is difficult to judge whether the insecticidal efficacy comes from azadirachtin itself or from the detergent added to emulsify this product. Considering the relatively high amount of detergent added, it is likely that the larvicide effect resulted more from the physical impact of the oil than from its insecticidal properties. Although the environmental impact was dramatic, a similar impact is also obtained with a non-insecticidal oil. Therefore, the impact of detergent alone on anopheline larvae, under field conditions, should be investigated in order to draw practical conclusions and make recommendations.

Neem oil application for wall spraying is not recommended because of its dark green colour, oily nature and the large quantity necessary for this application.
Larvicides tested Neem oil was purchased from Plasma Power Private Limited, India. Two locally produced insecticides were also tested: Temephos and Chlorosol. Temephos consists of 50% Temephos, 11% berol, and 39% emulsifiers and white kerosene. Chlorosol consists of 20% chlorpyriphos methyl, 20% fenitrothion, and 60% emulsifiers and solvents.

Laboratory study The ready-made detergent, FEBA, a dishwashing liquid, proved to be the best emulsifier for neem oil at 20% of neem's concentration.

Laboratory bioassay The susceptibility tests of anopheline (laboratory and field strains) and Culex pipiens field strains towards neem oil, Temephos and Chlorosol were carried out in the laboratory. Moreover, the susceptibility of field strains of anopheline larvae collected from El-Henawy village against neem oil and anopheline larvae collected from Abheit El-Hagar village against Chlorosol were evaluated after 3 months of biweekly application of the tested substances. Laboratory strains of anopheline larvae were selectively pressured against neem oil for 5 generations and their susceptibility towards neem oil was recorded. The susceptibility test of the field strains of Daphnia magna and Gambusia affinis against neem oil, Temephos and Chlorosol were carried out, and the toxicity of laboratory mice against neem oil was studied.

Efficacy of neem oil, Temephos, and Chlorosol as anopheline larvicides El-Henawy village was divided into 4 sectors, and in each sector, 5 different water bodies were chosen and treated with the above-mentioned substances and 5 water bodies were left without treatment as the control group. Larval density was determined prior to treatment, and on days 1, 2, 3, 7, 14 and 28 of post-treatment in accordance with WHO guidelines, 1975.

Field efficacy of implementation of neem oil as an alternative larvicide Six selected water bodies in El-Henawy village were treated biweekly with 5% neem oil emulsified in 1% ready-made dishwashing detergent, while those of Abheit El-Hagar village were treated according to the schedule of the Ministry of Health and Population of biweekly back-spraying with Chlorosol at 10 ml Chlorosol per 10 litres of water covering 100 square metres of surface area. Seven rounds of biweekly application continued from 30 June until 21 September.

Larval densities were monitored prior to application and weekly post-application until the end of the above schedule and four weeks thereafter (30 June through 19 October). Larval density was determined according to WHO guidelines, 1975.

Parallel adult density was determined to study the impact of larviciding on adult density. In each village a group of 10 houses and another group of 10 animal sheds were randomly selected, and mosquitoes were collected by the spray-sheet collection method using pyrethrum solution.

Residual effect of the neem oil Five houses were randomly selected in El-Henawy village for determining the impact of the residual action of neem oil on adult mosquito density. Five percent neem oil plus 1% ready-made dishwashing detergent (500 ml neem oil + 100 ml ready-made dishwashing detergent for a 10-litre back sprayer) was used to cover 250 square meters of indoor walls and ceiling. Adult mosquito density was monitored pre-application and biweekly for seven rounds (14 July through 21 October). Mosquitoes were collected, identified and their density was determined.

Main study findings Ready-made FEBA detergent is better than any other tested emulsifier for its efficiency, reproducibility of results, toxicity to the environment and cost. There was no significant difference in the susceptibility of laboratory versus field strains of anopheline larvae towards crude neem oil. Moreover, the field efficacy of neem oil as an anopheline larvicide is comparable to the other tested traditional larvicides, but it is efficient for only 2 weeks compared with 4 weeks for the other larvicides. Also, neem oil is as efficient as the Chlorosol in reducing the anopheline adult density and the number of adult-infested sites.

This study also showed that vegetable oil is almost as toxic as neem oil towards Culex pipiens and Daphnia magna and other tested non-target organisms. This might be due to the low concentration of azadirachtin in the crude neem oil (1570 ppm) and the high concentration of the detergent, and therefore, the main mode of action of neem oil in this case is through its physical properties rather than its azadirachtin contents. Using traditional mineral oils as larvicides might bring the same results.

The preliminary toxicological impact of crude neem oil on male mice did not prove any significant changes in body weight gain, liver function or blood parameters.

The cost of quantity of the neem oil-detergent emulsion required to control anopheline larvae in a certain area is 2 to 5 times greater than the cost of Chlorosol or Temephos to control the same area.

Conclusions and recommendations The results of this study cannot support large-scale use of neem oil as an insecticide unless justified by acute insecticide resistance problems. Before recommending its use as an alternative larvicide, there is a need to study the impact of long-term field application and its toxicological impact. The larvicidal effect of neem oil should also be compared with other fuel oils.
Malaria vector control through three different larviciding strategies

Oman
Wilayat Barka, South Batinah Region

Study period:
May 2001–January 2002

Small Grants Scheme
(SGS) 2000 No. 72

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Abstract
A field study was carried out in South Batinah Region to evaluate the impact of 3 larviciding-based strategies of vector control. The evaluation covered 2000 breeding sites in this region and continued over 27 weeks. The first strategy was to use half the amount of Abate (0.5 ppm) weekly, instead of the conventional weekly dose of 1 ppm currently used in the National Malaria Eradication Programme. The second approach was to apply the full dose (1 ppm), but fortnightly. The third method was to search for vector larvae and treat only the breeding places of anopheline larvae. The first method was found to be as effective as the full dose and the second approach was the least effective larvicidal method.

Conclusion
Compared to the full dose of Abate currently used in the control programme of the Ministry of Health, the half dose of Abate (0.5 ppm) was proved to be a more cost-effective and environmentally friendly larvicidal-based vector control method. It is much more effective than applying a full dose fortnightly or treating only larval-positive breeding sites. This vector control method could be adopted in malaria endemic countries.

Background
The National Malaria Eradication Programme in Oman has achieved excellent malaria control through case management and vector control, including larval control, using chemicals. This study reports the results of comparing different larvicidal strategies for vector control in the field.

Materials and Methods
Oman consists of 10 administrative regions, each divided into wilayats. At the wilayat level, malaria units are responsible for malaria eradication activities. The area under each unit is demarcated into daraks for all operations. Centrally, the Directorate of Environmental Health and Malaria Eradication supervises the national programme.

The study was carried out in 5 coastal daraks of the malaria-free wilayat of Barka in the South Batinah region. Geographic reconnaissance was updated for the vector breeding locations by physical verification. Final maps indicating details of the work were then prepared. Meteorological data were collected from Seeb Weather Station. The pH value in each darak was also recorded.

The intervention
The intervention consisted of using several different strategies for applying Temephos insecticide. Some daraks were treated using the existing standard method, but applying only half the standard weekly dose of Temephos; others were treated with the normal 1 ppm dose of Temephos, fortnightly; and in the third group, only positive larval breeding sites were treated. One darak was left without intervention to observe the natural conditions and as a control for comparison to the above-mentioned strategies.

Supervisory teams closely directed the sprayers and regularly delivered the daily programmes for spraying. All daraks were visited weekly to assess the impact of the treatment strategy on larva control.

Conclusions and implications of the study
- A half dose of Abate (0.5 ppm) is more cost-effective compared to a full dose, with less harmful effect to the environment. Hence this method could be applied on a wide scale in endemic countries.
- In countries targeting elimination, it is recommended to stratify the country according to receptivity and vulnerability to malaria, and apply the tested larvicide wherever there is a risk of resuming malaria.
- The frequency of positive breeding sites for the vector in this region ranged from 4% to 6.6% in June and increased during the months of August to December.
- *Anopheles culicifacies* and *An. stephensi* are the only species identified in this region.
Vector susceptibility to Temephos

A pre-intervention baseline evaluation of the vector susceptibility to Temephos was performed using WHO standard methods for susceptibility testing. Larval density tests were also conducted.

Main study findings

Baseline pre-intervention survey: June 2001
There was no significant difference between the tanks regarding the presence of the vector during this period (ranging from 4 to 6.6%). The only species identified were *An. culicifacies* and *An. stephensi*, and the former was much more prevalent (vector density: 10-25/100 dips for *An. culicifacies* compared to 0-7/100 dips for *An. stephensi*). Vector susceptibility tests to Temephos were also conducted in each darak.

Intervention period: 30 June-December 2001

Control daraks

Vector breeding in this area markedly increased during the period August-December, reaching 20-50 larvae/dip without application of any vector control measure.

Weekly half-dose treatment with Temephos

In this intervention, there was a significant decrease in vector density in the treated daraks, with vector numbers declining from an initial 30 larvae/dip to none after 4 cycles, and this was maintained till the end of the study.

Fortnightly normal dose of 1 ppm

Although there was a significant decrease in vector density with this intervention, it was not 100% effective.

Weekly breeding checking and treating of positive sites

While this intervention was more effective in reducing vector density compared to the fortnightly normal dose of 1 ppm, it was less effective compared to the weekly half-dose treatment with Temephos.

Vector breeding sites

Twenty percent of potential breeding sites were positive for the vector during the study period. However, there was a significant difference between sites regarding the presence of the vector. Interestingly, repeated identification of larvae was reported up to 10 times, indicating a tendency of the vector to use the same breeding site or "habitat" in this situation.

Larvicidal strategy

Multivariate logistic regression analysis showed that weekly half-dose treatment with Temephos provided the most protective effect against positivity of breeding sites. This was adjusted for the effect of temperature and pH value of the daraks.

Vector susceptibility tests

There was no significant difference before and after intervention regarding vector susceptibility to Temephos.

Cost-benefit analysis of different strategies

The cost for larvicide was reduced by 50% using weekly half-dose applications of Temephos, which was proved to be as effective as using the full dose, and was the most effective tested strategy. The fortnightly cycle was the most economic method, but was the least effective.

Conclusions and recommendations

A half dose of Abate is a cost-effective larvicidal-based vector control that could be applied on a wider scale in endemic countries. However, in countries targeting elimination, it is recommended to stratify the country according to receptivity and vulnerability to malaria, and apply the tested larvicide where there is a risk of resuming malaria. Testing the half dose of Abate for a two-week cycle is also recommended to provide a further reduction in cost and harm to the environment.
**Abstract**

This study was conducted in an onchocerciasis endemic village in southern Darfour state. Male individuals aged 15-60 years were interviewed regarding itching and musculo-skeletal pains. Individuals with skin lesions were examined for dermatological signs of onchocerciasis. The degree of severity and morbidity of onchodermatitis skin lesions were assessed and graded. Ninety patients with moderate or severe onchodermatitis were selected and randomized into one of the following ivermectin treatment schedules: 3, 6, or 12 monthly treatment. Ivermectin was administered in single oral dose of 150 µg/kg. Ivermectin was distributed to the rest of the villagers as part of the first ivermectin mass distribution programme conducted in this area.

The three study groups were followed every 3 months and compared regarding the time needed for disappearance, improvement or recurrence of symptoms, or changing in the severity of the skin lesions.

**Results**

Itching whether troubling or severe was the commonest symptom reported by all patients with moderate or severe onchodermatitis. Both quarterly and biannual ivermectin treatment lead to significant reduction in prevalence of itching while 12 months treatment resulted in partial decline in its intensity. Moreover, quarterly and biannual schedules resulted in significant reduction in the prevalence and intensity of Acute and Chronic Papular Onchodermatitis, and Lichenified Onchodermatitis. Muskuloskeletal complaints was the prevalent symptom in onchodermatitis. Both the joint, bone, and backache showed good response to ivermectin treatment irrespective of the treatment schedules. Onchocercal depigmentation was the only skin lesion not responding to ivermectin treatment, however, ivermectin prevented the development of new skin lesions.

**Conclusion**

There was no significant difference between biannual and quarterly ivermectin treatment schedules regarding the improvement of signs and symptoms of onchodermatitis. The 6 monthly ivermectin treatment schedule proved to be a cost-effective schedule for controlling moderate or severe onchodermatitis in endemic areas.

**Background**

Onchocerciasis has long been associated with a high incidence of detrimental effects on socioeconomic development and public health in endemic areas. Ocular complications of onchocerciasis and the response to treatment with ivermectin, both have been extensively investigated. Other manifestations related to infection with *O. volvulus* such as onchodermatitis and musculoskeletal pains have received less attention. Only recently the psycho-social importance of skin disease became evident. Symptoms such as itching can lead to sleeplessness, fatigue, weakness and less capacity to work.

There is enough evidence that skin lesions and itching are reduced with ivermectin. Yet, the optimum schedule of treatment is not worked out especially. Muskuloskeletal symptoms due to...
Onchocerciasis could have a major socioeconomic impact especially in farmer communities, where it was found to be the major cause of morbidity and loss of working days. However, little is known about the response of these complaints to ivermectin. An earlier observation by the research team following mass drug administration of ivermectin indicated that this complaint improved at the follow-up visit. This study was conducted aiming at determining the optimum treatment interval with ivermectin for controlling onchocerciasis skin disease.

**Materials and methods** A village in Radom focus, Mirarie, southern Darfur sate, where ivermectin has not been previously distributed, has been selected. The prevalence of onchocerciasis was 35% using the method of Rapid Epidemiological Mapping of Onchocerciasis (REMO). The village census was performed and all male individuals aged 15-60 years were enrolled in the study.

The format developed for WHO Multicountry study of the economic impact and social cost of non-ocular onchocerciasis was used. Symptoms of itching and musculo-skeletal pains were recorded and graded as (0/1/2/3) according to the ability of the patient to carry out the respective activity such as to work or sleep. The dermatological signs of the disease were determined. Individuals with skin lesions were carefully examined for dermatological signs of onchocerciasis. The degree of severity and morbidity of onchodermatitis skin lesions were carefully assessed and graded into four grades. Ninety patients with moderate or severe onchodermatitis were selected and randomized using stratified random sampling into three groups of 30 patients each. Stratification was based on different grades of skin lesion severity. The lesions were also mapped and photographed. Each of the three study groups were randomly allocated to one of the following ivermectin treatment schedules 3, 6, or 12 monthly treatment. administered in a single oral dose of 150 µg/kg. Ivermectin was distributed to the rest of the villagers as part of the first ivermectin mass distribution programme conducted in this area.

The three study groups were followed every 3 months using the same initial methodology.

Data analysis consisted of determination of time needed for disappearance, improvement or recurrence of symptoms, or changing in severity of the skin lesions.

**Main study findings**

Sixty-three out of the 90 patients had completed the study. There was no statistically significant difference between the three study groups regarding their response to the three treatment schedules. Itching was the commonest complaint manifested by all the study population (63 patients). The intensity of itching varied between troubling (55%) or severe (40%). The best response of itching to ivermectin treatment was obtained 6 months following the initial dose. Annual treatment resulted in reduction of the patient suffering causing a decline in the severity of itching but did not lead to significant decline in the prevalence of this symptom. Following treatment, both 3 and 6 month ivermectin schedules resulted in 90 % reduction of the prevalence of itching 12 months post treatment, with no significant difference between both schedules regarding prevalence or intensity of this symptom.

Musculoskeletal complaints such as joint, bone or backache were reported in 84% and 92% of the study population, respectively. There was significant reduction in the prevalence and intensity of these complaints following treatment, with no significant difference in the efficacy of the three treatment schedules.

Acute Papular Onchodermatitis (APOD) was reported in 79% of the study population. The biannual and quarterly ivermectin treatment schedules resulted in significant decline in the prevalence of APOD from 61% and 90% respectively to 15% each, at 12 months post treatment. Up to 6 months post treatment schedules had comparable results. However, significant changes were evident in both the biannual and quarterly treatment schedules during subsequent follow-up visits.

Regarding Chronic Papular Onchodermatitidis (CPOD), it accounted for 68, 81, and 85% in annual, biannual and quarterly ivermectin treatment schedules. The annual treatment schedule did not lead to any significant reduction in the prevalence of the skin lesions. The quarterly and biannual ivermectin treatment schedules had effectively reduced the prevalence of the lesion, while the three schedules had reduced the severity of the lesion. This was manifested by disappearance of excoriation with or without super infection.

Lichenified Onchodermatitidis (LOD) was reported from 70-73% of patients in the three groups. There was no significant difference between the three schedules regarding the prevalence or intensity of infection. However, the biannual and quarterly schedules recorded comparable results at 9 and 12 month follow-up.

It is worth mentioning that depigmentary skin changes were the most disfiguring and stigmatizing lesion of onchodermatitis. This was reported in 40 to 54% of the studied groups and did not record a significant improvement with treatment. Few patients who presented with very early brown pigmentation reverted to normal. Patients initially presenting with late brown pigmentation gradually progressed to leopard skin which was irreversible.
Schistosomiasis Control

Evaluation of an integrated approach of schistosomiasis control (chemotherapy and snail control) in a resettlement area, west of Alexandria, Egypt

Abstract

In a previous study carried out by the author between 1988 and 1991, a schistosomiasis control programme was undertaken in Mariout, west Alexandria, an area reclaimed from the desert. The baseline situation of schistosomiasis was established in a sample of 3 villages with a total population of 6577 individuals. The prevalence of Schistosoma mansoni in the 3 villages was 22%, 28% and 40%. A survey of the water channels revealed that 60%-80% of them dried up between irrigation rounds. Biomphalaria alexandrina was detected in channels permanently containing water. The proportion of infected snails was unexpectedly high (7.5%, 15.8% and 17.3%). In 1989, all positive cases were called for treatment with a single dose of praziquantel. This was followed by an abrupt drop in the disease prevalence of infection to 18.5%, 14.9% and 15.4%. In 1990, snail control using 70 ppm of the dry form of the plant molluscicide Ambrosia maritima (damsissa) was applied. This led to a 90% drop in the snail population and in the number of infected snails. Schistosomiasis prevalence showed a further slight decline, with disease prevalence rates becoming 17%, 14% and 12%. No control activities were undertaken thereafter. The objectives of the present project were to determine the level of schistosomiasis in the area, 3 years after the cessation of control measures, to find an explanation for cases converting to positive, and for cases reverting to negative.

Results

Revision of the census data in 1993 demonstrated an overall net increase of the population (including new settlers) by 14.6% as compared to the census of 1988. The prevalence of S. mansoni was still on the decline and the rates had become 13.6%, 14.4% and 9.0%. A survey of the water channels did not reveal any significant changes in the total length of the water channels or of the infected ones. The water network had not been modified. The snail population had increased compared to the baseline findings, but the proportion infected was very low (0%, 0.5% and 0.9%). A study of the conversion indicated that the yearly incidence was 11%, whereas it was 14% before control. It was found that 20% of converting cases reported working outside the area, 21.5% were new settlers and the remaining 60% were probably infected locally. A study of reversion revealed that only 7% of positive cases had been treated in the previous 3 years. It was found that 30% of cases were missed by the Kato-Katz technique (2 slides). The remaining 63% could be considered self-cured during the 3-year period. This finding was explained by the fact that the mean life span of the S. mansoni adult worm is 3-4 years. Accordingly, in the absence of re-infection, 20-30% of the infected individuals lose their infection every year.

Conclusion

An integrated control programme, applied once, could control schistosomiasis in both the human and snail hosts for a period of 4 years. However, the increase in snail population points to the possibility of reaching the original level of infection. These findings generally call for application of integrated control measures every 3 years.

Conclusions and implications of the study

- Integrated control measures for schistosomiasis could be successfully achieved by a single dose of praziquantel (40 mg/kg body weight) and snail control using 70 ppm of the dry form of the plant molluscicide Ambrosia maritima (damsissa).
- The Kato-Katz technique (2 slides) records a considerable percentage of false-negative cases (30%) when used in low infection intensity (fewer than 100 epg).
- The mean life span of the S. mansoni adult worm is 3-4 years. Accordingly, in the absence of re-infection, 20%-30% of the infected individuals lose their infection every year. However, the increase in snail population points to the possibility of reaching the original level of infection with population movement from areas where no control measures were implemented. The application of integrated control measures every 3 years is therefore recommended.

Publications


Background

Mariout, 40 km to the west of Alexandria, consists of land reclaimed from the desert, and irrigated by El-Nubarieh canal. An
integrated schistosomiasis control programme was implemented in this region during the period of 1988-1991. In the baseline survey, the prevalence of *Schistosoma mansoni* in 3 villages in this area was 22%, 28% and 40%. The proportion of infected snails was unexpectedly high (7.5%, 15.8% and 17.3%). Positive cases were treated with a single oral dose of praziquantel. This was followed by an abrupt fall in the disease prevalence of infection to 18.5%, 14.9% and 15.4%. Snail control was performed by applying the plant molluscicide *Ambrosia maritima* (Damsissa) to all infested water channels. One year later, the control programme was evaluated and was considered successful. The present study was conducted by the same research team, aiming at determining the prevalence of schistosomiasis in this area, 3 years after the cessation of control measures.

### Materials and methods

Following the same methodology as the initial study, 3 previously surveyed villages (Orabi, El-Gazayer, and Palestine), out of a total of 26 in the area, with a total population of 6577 individuals, were selected. Maps of the houses and water channels around the villages were prepared and a census of the population was recorded. A stool survey of all villagers was performed using the Kato-Katz (2 slides) methods. Stool specimens were obtained from 191 individuals with a low intensity of infection. Each specimen was examined by Kato-Katz smear technique (2 slides).

Snails were collected, counted, crushed and examined under the dissecting microscope for trematode infections. Villagers were interviewed according to a questionnaire including information regarding population movements, such as having an occupation outside the village or being a new settler in this area, and history of anti-schistosomal treatment during the initial study. Stool specimens were obtained from 191 individuals with a low intensity of infection. Each specimen was examined by Kato-Katz smear (2 slides), formol ether concentration and simple sedimentation.

### Main study findings

The revised census indicated a 14.6% increase in the population. It was observed that a proportion of the population moved to urban areas, while new settlers added 26% to the original population. The environmental conditions in this area were unfavourable for the snail habitats; the soil is sandy with limited water supply, and accordingly, the majority of water channels becomes completely dry between irrigation rounds. However, *B. alexandrina* was identified in the few canals and drains containing water all year round or that dried for only short periods. The snails were collected from the 3 villages throughout the year, with their numbers peaking in April. El-Gazayer village recorded the highest frequency of snails, followed by Orabi and Palestine. Compared to the baseline survey, there was a 2-fold increase in the number of snails in El-Gazayer and Orabi villages, and a significant reduction (50% reduction) in the number of snails collected from Palestine village. In spite of the overall increase in the snail population, the proportion infected was very low (0%, 0.5% and 0.9%). The study of the water network revealed that in Orabi, the stretch of the canal carrying water increased together with the infested channels. In El-Gazayer, there was a reduction in the length of canals and the number of harbouring and infected snails. In Palestine, in spite of the increase in the amount of water, the length of infested channels had not changed and no canals were infected.

The prevalence and intensity of infection in the 3 villages were still declining one year after the application of the control measures in Orabi and Palestine, thereby indicating a decrease in the transmission potential in these 2 villages; the prevalence dropped to 13.6% and 9.0%, respectively, and the intensity from 52 to 34 epg, and from 49 to 32 epg, respectively. On the other hand, there was a decrease in the mean egg count of positive cases in El-Gazayer while the prevalence was maintained at 14.4%. Infection in children younger than 5 years of age was significantly reduced to 1.2% compared to 10% in 1988 and 2.7% in 1991.

**Study of conversion (negative to positive)**

This was mainly related to working outside the area and to new settlers.

**Study of reversion (positive to negative)**

One hundred forty five positive cases in 1991 were negative in the present study, 7% reported treatment during the previous 3 years, and 30% were missed, therefore, the remaining 63% could be considered self-cured during the 3-year period. This finding was explained by the fact that the mean life span of the *S. mansoni* adult worm is 3-4 years. Accordingly, in the absence of re-infection, 20%-30% of the infected individuals lose their infection every year.

It was also found that 30% of cases were missed by the Kato-Katz smear technique (2 slides).

### Conclusions and recommendations

An integrated control programme, applied once, could control schistosomiasis in both the human and snail hosts for a period of 4 years if the same conditions are maintained (no re-infection). However, the increase in the snail population points to the possibility of reaching the original level of infection, especially considering the significant population movement. These findings generally call for the application of integrated control measures every 3 years.
**Abstract**

The prevalence of schistosomiasis in Ma'rib Province, and the distribution and infection rate with Schistosoma cercariae of the snail intermediate hosts in Ma'rib Dam were investigated. A total of 2650 urine and 2023 stool samples were examined in the central hospital of Ma'rib Province, Republic of Yemen.

**Results**

The overall prevalence of *Schistosoma mansoni* was 35.1% and of *S. haematobium* 18.3%, while 1.2% of the infected subjects had mixed infections. The infection rate for both urinary and intestinal schistosomiasis was higher in males than in females. The highest prevalence rates were observed in males and females aged 16 to 20 years. All infected persons were treated with a single dose of 40 mg/kg praziquantel.

**Background**

Schistosomiasis due to both *Schistosoma haematobium* and *S. mansoni* is endemic in Yemen. According to a rough estimation made in 1971 and confirmed by further studies done through 1982, more than one million people of Yemen have one or both types of infection. In view of the lack of information regarding schistosomiasis conditions in Ma'rib Province, where the largest dam in the country is located, the present study was conducted to assess the prevalence of urinary and intestinal schistosomiasis in the inhabitants of Ma'rib Province, the distribution of the snail intermediate host in 3 localities around the Ma'rib dam and their infection rate with Schistosoma cercariae.

**Conclusions and implications of the study**

- The prevalence of *S. mansoni* and *S. haematobium* infection in Ma'rib governorate was 35.1% and 18.3%, respectively, and 1.2% of the infected individuals had mixed infection.
- The highest infection rates occurred in the age group of 16 to 20 years, with higher rates in males, which was attributed to their increased exposure during work in the field, swimming, bathing, or fishing in infested water.
- The *Biomphalaria arabica* snail was more widespread than *Bulinus truncatus* and the rate of infection of Biomphalaria with Schistosoma cercariae was higher than that of Bulinus, indicating that the types of snail breeding habitats are the main determinants of the species of parasite infecting inhabitants of different areas.
- Schistosomiasis control was performed by praziquantel administration to all infected cases and by snail control using Bayluscide. Destruction of all infested locations around the dam, proper disposal of sewage to decrease human exposure to infected snails, mass treatment and educational programmes to increase awareness of the problem were recommended.
in December 1986. Three localities (the main gate of the dam, Diversion A and Diversion B) were investigated for the presence of snails. Each site was inspected twice each season for 2 years. Snails were searched through examination of submerged and emergent plants. A minimum of 1 hour was spent at each site during each visit. Snails found were placed in wide-mouthed screw-cap containers and brought alive to the laboratory for identification. The method of Teesdale et al was used to examine the infected snails. Snail control with Bayluscide was performed throughout the canals, especially those with a higher probability of water contact.

Evaluation of the control measures was not performed by the research team.

Main study findings

The prevalence of S. mansoni and S. haematobium infection accounted for 35.1% and 18.3%, respectively. In both urinary and intestinal schistosomiasis, the highest rates of infection occurred in the age group of 16 to 20 years, and it was higher in males than in females, though this difference was not significant. Fourteen subjects (1.2% of the infected individuals) had mixed infection. Bulinus truncatus, the intermediate host of S. haematobium, was found in 2 habitats (the main gate of the dam and Diversion B) out of the 3, while Biomphalaria arabica, the intermediate host of S. mansoni, was found in all the 3 inspected habitats.

The rate of infection of Biomphalaria arabica with S. mansoni cercariae was 1.65% while the rate of infection of Bulinus truncatus with cercaria of S. haematobium observed during the survey was 1.3%. This was consistent with reports from Taiz province, that Biomphalaria arabica was more widespread than Bulinus truncatus and the rate of infection of Biomphalaria with Schistosoma cercariae was very high in comparison to that of Bulinus [1].

It seems that the density of both Biomphalaria arabica and Bulinus truncatus is in continuous increment. In 1985, a team from World Health Organization (WHO) made an extensive study to assess the potential impact of Ma’rib dam on the health conditions in Ma’rib province, but they did not find the snail hosts for schistosomes [2]. In 1990, Arfaa [3] found both snails in two ponds immediately down stream of the main canal of the dam. In 1991, Nagi [4] found that canals of irrigation systems of Ma’rib dam were heavily infested with snails, and he stated that, in some places, shells covered water plants like salt. In view of what is mentioned above, the high rate of infection recorded among the inhabitants of Ma’rib province is not surprising.

References


Conclusions and recommendations

The differences in the prevalence rates of the two Schistosoma species may be attributed to the relative prevalence of the species of snail intermediate hosts transmitting them. The higher infection rate among males was attributed to their increased exposure during work in the field, swimming and bathing, or fishing in infested water. Furthermore, the types of snail breeding places and their suitability for the breeding of Bulinus or Biomphalaria snails are the main determinants of the species of parasite infecting inhabitants of different areas. It was recommended to discover and destroy all infested locations around the dam. Proper disposal of sewage to decrease human exposure to infected snails, mass treatment and educational programmes to increase awareness of the problem to control the disease, were also recommended.
Control of snail intermediate host of schistosomiasis using different molluscicides

Egypt
rural Alexandria

Study period:
January 1997–March 1998

Small Grants Scheme
(SGS) 1996 No. 49

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Abstract
Four water courses in Abis, rural Alexandria, with the same conditions and snail density were chosen for this study. All methods were applied simultaneously in May. Snails were collected monthly to evaluate the malacological effect. A single application of 100 ppm dried *Ambrosia maritima* decreased the snail density significantly within one month. The snail density was maintained at a very low level until the end of the year. Fresh *Azolla pinnata* applied at a dose of 280 ppm lowered snail density to a level intermediate between *Ambrosia maritima* and Bayluscide. The snail population began to build 5 months later. Bayluscide applied once at a dose of 1 ppm significantly lowered the snail density. This lowering was maintained for 4 months after which the snail density began to build up and remained high until the end of the study period. Clearing of vegetation was followed by an abrupt lowering of snail density, and this was maintained for a period longer than that following Bayluscide and Azolla.

Publications

Background
The successful control of schistosomiasis should be based on an integrated approach which includes the control of the intermediate snail host. For this reason, there is a continuous need for the development and evaluation of new, inexpensive, but highly effective molluscicides. Many developing countries are reluctant to embark on chemical snail control programmes, using costly synthetic compounds from industrialized nations. National and international institutions are currently giving increasing attention to the study of plant molluscicides in the hope that they may prove to be cheaper and more readily available for snail control compared to synthetic chemicals.

In Egypt, the annual herb, Damsissa (*Ambrosia maritima*), is a biological moluscicide, and could be a part of the ecosystem. It was reported that this plant has lethal effects on snails and their eggs due to its active moluscicide components, mainly Ambrosin and Damsin.

The Azolla plant is a symbiotic algal association that grows on the water surface. It is widespread in the western hemisphere as well as in Egypt. This plant plays an important role in the process of nitrogen fixing in the soil and as food for domestic animals; it is also a desirable organic fertilizer in rice fields. This plant lowers the survival, growth and fecundity of *B. alexandrina* snails.

Bayluscide is still recognized as the most effective chemical molluscicide, and it is predominantly used in snail control programmes. However, the cost of chemical molluscicides is soaring to such an extent that governments are reluctant to include their purchase in budgets. Clearing vegetation is one of the important physical methods of snail control. The present study aimed at evaluating

Conclusions and implications of the study

- A single application of Bayluscide at a dose of 1 ppm succeeded in lowering the snail density for a short period of time, not exceeding 3 months. On the other hand, this method is costly and was proved to be toxic for aquatic organisms such as fish.
- A single application of *A. maritima* at a dose of 100 ppm considerably reduced the number of snails. The snail population remained low for at least 7 months.
- The snail density decreased after a single application of *Azolla pinnata* (280 ppm) on *Biomphalaria alexandrina* snails. The number of snails was maintained at a low level for 4 months, then increased.
- Clearing of vegetation was followed by the abrupt lowering of the snail density, and this low level was maintained for 8 months.
- It was concluded that the best results for control of *B. alexandrina* could be obtained by clearing vegetation together with the application of Damsissa.
different methods of B. alexandrina snail control, namely chemical (Bayluscide), biological (plant molluscicides, Ambrosia maritima and Azolla pinnata), and physical (clearing of vegetation) means.

Materials and methods

This study was conducted in Abis 10 (Esbet El-Matar), 10 km west of Alexandria. Water courses chosen for this study were based upon: the presence of a significant number of B. alexandrina snails, their similarity in size and conditions, being equally distant from the village and with comparable human activities, water volume, pH, salinity, and presence or absence of vegetation. Twenty equidistant sampling stations were determined for each water body. The snails were collected by a wire net with 3 adjacent dips every 25 metres.

Molluscicides were applied during May 1997 as follows:

Chemical molluscicide: Bayluscide was applied at a dose of 1 ppm in the chosen drain using a drip feed technique with a drain dispenser that delivers a constant flow of the molluscicide.

Plant molluscicides: A. maritima was obtained in its dry form from Aswan and applied at a dose of 100 ppm. The dry Damsissa plant was applied in weighed nylon mesh bags, each contained 1/2 kg. The bags were distributed in alternation along both bank sides. A. pinnata was collected in its fresh form from different water courses outside the area. The fresh plant was applied by dispersal in alternation along both banks. The concentration of the plant was 280 ppm W/V. The land owner was asked to remove the vegetation from the fourth drain by the usual manual method. Snail collection was repeated using the same standard technique, 2 weeks after application of the control method, and then monthly until the end of the study.

Main study findings

Bayluscide application at a dose of 1 ppm led to a rapid decrease in snail density that was maintained for 3 months. This was followed by a rapid increase in the snail population, and the snail density remained high until the end of the study. Thus, a single application succeeded in lowering the snail density for a short period of time; then, it is likely that the chemical was washed away with the flowing water, and the environment became suitable again for the snails. Other main drawbacks appear to be its high price and its adverse effects on non-target organisms, particularly fish, at the concentrations used to control the snails.

The malacological data indicated that control of B. alexandrina was successful in the water courses which were treated with A. maritima. A single application of 100 ppm of the plant considerably reduced the number of snails. The snail population remained low for the rest of the year. This prolonged action may be due to the fact that the dried plant was fixed in situ and was not washed away with the flowing water. Furthermore, the plant was not toxic when used at the molluscicidal concentration.

This study revealed that the snail density decreased after a single application of Azolla pinnata (280 ppm) on the B. alexandrina snails. The number of snails was maintained at a low level for 4 months, then their numbers increased. Compared to Bayluscide, the total number of snails collected was lower after Azolla application, while compared to Damsissa it was higher. The clearing of vegetation was followed by an abrupt lowering of the snail density that continued for 8 months. The decrease in snail density was maintained for a longer period, longer than that which followed the application of Bayluscide or Azolla. This may have occurred because the snails were deprived of their food and shelter, and were not subsequently able to repopulate the water course in short period of time.

It was concluded that the best results for control of B. alexandrina would be obtained by clearing vegetation together with the application of Damsissa.
Abstract
An intervention study was conducted in Khemir, northern Sana’a, whereby integrated control of urinary schistosomiasis was performed by means of chemotherapy and health education. The study also aimed at investigating the validity of reagent strips as a rapid diagnostic tool, and their cost-effectiveness together with visible haematuria for rapid screening of **Schistosoma haematobium** infection. Houses and schools in the study area were randomly selected. A baseline survey was performed using a questionnaire and urine analysis. A total of 8540 individuals were treated with praziquantel 40mg/kg body weight, single dose. Weekly health education classes were held in schools where health education posters were distributed. Other health education sessions were also held in schools for non-enrolled children and their parents. A post-intervention survey was also performed by questionnaire and urine analysis.

Results
There was a significant decrease in the prevalence of infection 14 months post-intervention. The overall prevalence of **S. haematobium** infection dropped from 58.9% to 5.8%. Furthermore, there was a significant decrease in the frequency of heavy infections from 40% to less than 20% of infections. There was no significant difference between males and females, nor between literate and illiterate individuals regarding the prevalence or intensity of infection. Health education sessions resulted in a significant decrease in the frequency of contact with water sources and more adherence to preventive measures.

Conclusion
Reagent strips and visible haematuria could be cost-effective screening methods for the disease only if applied in remote areas with limited accessibility to health services. However, reagent strips are far less reliable diagnostic tools compared to microscopic urine examination.

Background
Schistosomiasis has been a public health problem in Yemen for the last 2-3 decades. Its endemicity together with other intestinal parasites has been frequently reported. However, there have been no previous reports regarding the situation in Khemir or integrated methods developed for schistosomiasis control in the whole country. The present study was therefore conducted to evaluate the impact of integrated surveillance and control methods on the burden of schistosomiasis in this area, as well as studying the validity of using reagent strips as a rapid diagnostic tool, and its cost-effectiveness, together with visible haematuria, for rapid screening of the **S. haematobium** infection.

Materials and methods
Setting Al- Khemir, located 90 km north of Sana’a, is an agricultural area depending on rain and ground water for irrigation and domestic use.

Design This was an intervention study targeting community and schoolchildren.

Baseline community survey A list of the houses in the study area (1000) was prepared and a random sample of 100 houses (863 individuals) was selected. Each individual was interviewed using a questionnaire and was subjected to urine examination.

Baseline school survey A sample of 20% of schoolchildren (287
schoolchildren), randomly selected from the 14 schools of the area was included in the study. These children were also interviewed using a questionnaire and were subjected to urine examination.

**Intervention** The integrated methods of schistosomiasis control consisted of chemotherapy for *S. haematobium* infection and health education. A total of 8540 individuals were treated with praziquantel 40mg/kg, single dose. These were schoolchildren, non-enrolled school-age children, and preschool children and their parents. School teachers from all 14 schools in the district participated in a 2-day seminar. Weekly health education classes were held in schools where health education posters were distributed. Other health education sessions were also held in schools for non-enrolled children and their parents. They were educated about the disease and briefed about the study. They were also given detailed information regarding methods of health education for the different groups, chemotherapy for schistosomiasis and its dosage, and record-keeping. One teacher from each school was appointed as the coordinator for the control activities in his/her school.

**Community evaluation survey** (14 months post intervention) The houses in the study area (1000) were listed and a study sample of 100 houses (913 individuals) was randomly selected. Evaluation was performed via questionnaire and urine analysis.

**School evaluation survey** (14 months post intervention) Three hundred and twenty three schoolchildren, consisting of 20% of all schoolchildren in the area, were subjected to post-intervention evaluation via questionnaire and urine analysis.

**Validation of reagent strips** The reagent strips were compared to microscopic urine examination to determine their validity as rapid diagnostic tools for *S. haematobium*.

### Main study findings

The overall prevalence of *S. haematobium* infection was 58.9%. The age-specific prevalence was 48.3%, 61.3% and 69.3% among preschool, schoolchildren, and non-enrolled school-age children, respectively. The prevalence of infection was 55.2% among 16-40 years old individuals, compared to 42.9% in older individuals.

Regarding the intensity of infection, the majority of infections (60%) were light (50 eggs/10 ml urine) and 40% of infections were heavy. The frequency of light infections in children (15 years old) was significantly higher than heavy infections. By contrast, the frequency of heavy infections was significantly higher than light infections in the older age groups. There was no significant difference between males and females, nor between literate and illiterate individuals regarding the prevalence or intensity of infection. There was a significant decrease in the prevalence of infection 14 months post-intervention. The overall prevalence dropped to 5.8%, ranging from 4.9% among 6-15 year-old children to 7.8% among individuals aged 16-40 years. Furthermore, the majority of infections were light infections (81.1%), regardless of age or gender.

**Impact of health education** The frequency of contact with water sources significantly decreased from 95% and 98% to 9% and 88% in the community and school surveys, respectively. Similarly, adherence to preventive measures rose from almost none to 97% and 88% in the community and school surveys, respectively. Almost all the interviewed populations were treated and attended health education sessions (up to 97% chemotherapy and 99% for health education). They reported that they knew about the programme from the mobile team of the programme (90%), from the head of the community (88.1%), and a limited number (15.3%) reported school sessions as being their source of information. There was mutual satisfaction with the programme from those who implemented the programme as well as from its recipients. In fact, health education sessions were rated as very good or excellent by 85% of the studied population, and almost all community leaders and school teachers reported the smooth implementation of the programme.

**Validation of reagent strips** Compared to the parasitological examination of urine, the reagent strips recorded low sensitivity (56.5% and 56.95% in community and school surveys, respectively), low specificity (57.3% and 52.9%, respectively), limited positive predictive value (72.2% and 68.3%, respectively), and low negative predictive values of around 40% in both surveys. There was bad concordance between these reagent strips and parasitological examination of urine (Kappa = 0.1 in both surveys).

**Cost-effectiveness of screening by the reagent strips and visible haematuria** The screening methods greatly reduced the cost of the control programme in the study area (Wadiaa), which is a remote area with limited facilities.

### Conclusions and recommendations

An integrated school-based programme of chemotherapy and health education is an effective means to control *S. haematobium* in endemic areas. Reagent strips and visible haematuria could be used for screening the disease in remote areas with limited accessibility to health services, however, reagent strips are far less reliable diagnostic tools compared to microscopic urine examination.
Abstract
Schistosoma circulating antigens were used to indicate the infection intensity and to assess cure. An immunoglobulin G2a (IgG2a) mouse monoclonal antibody was used in a fast dot-enzyme-linked immunosorbent assay (ELISA; FDA) for rapid and simple diagnosis of schistosomiasis in the field. Seven hundred Egyptians were examined for Schistosoma mansoni and other parasitic infections. A rectal biopsy was done as a "gold standard" for individuals showing no S. mansoni eggs in their faeces. Egg counts were obtained by the Kato-Katz smear method. Specific anti-schistosome IgG antibodies were evaluated in sera by ELISA. Urine samples from the 700 individuals were tested by FDA for detection of the circulating antigen. The assay showed a sensitivity of 93% among 433 infected individuals and a specificity of 89% among 267 noninfected individuals. FDA showed the highest efficiency of antigen detection (91%) compared with the efficiency of antibody detection by ELISA (75%) and stool analysis (60%). In addition, FDA detected infected patients with 20 eggs/g of faeces. Also, the sensitivity of FDA ranged from 90% to 94% among samples from patients with different clinical stages of schistosomiasis. All the assay steps could be completed within 30 minutes at room temperature for 96 urine samples. The monoclonal antibody identified a 74-kDa antigen in different antigenic extracts of S. mansoni and S. haematobium and in the urine of infected individuals. In addition, a 30-kDa degradation product was identified only in the urine samples. On the basis of these results, FDA could be used as a rapid tool for the sensitive and specific diagnosis of Schistosoma infection.

Publications

Background
The dot enzyme-linked immunosorbent assay (ELISA) type of immunodiagnostic test has been reported for use in the detection of schistosomiasis. In the present study the sensitivity and specificity of circulating antigen detection in urine was evaluated by a newly developed fast dot-ELISA assay (FDA) and compared with those of standard traditional techniques for the rapid and simple diagnosis of human schistosomiasis in the field.

Materials and methods
Seven hundred individuals were subjected to full clinical examinations, stool, urine and blood examinations. A rectal biopsy was done as a "gold
PBS-T20 for 15 minutes on a shaker. After removal the filter was done with 2% (w/v) nonfat dry milk in PBS-T20 for 1 minute. Blocking of nonspecific binding sites on dried and then washed in 0.3% PBS-T20 for 1 minute. Then, the NC filter was air-dried and held at room temperature. The NC filter was washed in distilled water and soaked in a bath of PBS (pH 7.2), each for 1 minute. Then the filter was dried and kept in the dark. Positive controls for the assay were the affinity-purified antigen either from SWAP or from the urine of infected individuals and neat urine samples from infected individuals. Negative controls were urine samples from noninfected individuals. FDA allows a semiquantitative reading of the resulting coloured spot in case of antigen detection (i.e. a positive test result). The purple colour that was produced varied in its intensity from weak (1+ or 2+) to strong (3+ or 4+). Positive controls with these different colour intensities were used. A colourless spot was produced in the case of a negative test result. The resulting colour for the tested sample was then compared and related to the colour of one of the positive and negative controls with the naked eye.

**Main study findings**

FDA had a higher sensitivity (93%) than microscopic examination of eggs in stool (35%) and a higher specificity (89%) than anti-schistosomal antibody detection in serum by ELISA (56%). Moreover, FDA had sensitivities ranging from 90% to 94% for the different clinical stages of schistosomiasis. Also, it could detect the schistosome antigen in urine samples from patients with light infections of 20 epg of faeces. The circulating antigens were detected in individuals with low egg counts [2].

We can conclude that the FDA developed on the basis of an IgG2a MAb for the detection of Schistosoma circulating antigen excreted in urine proved to be a simple, rapid, sensitive, and specific enzyme immunoassay. The urine sample was used without any treatment, the assay needs no sophisticated equipment, and 96 urine samples could be run in about 30 minutes. In addition, all assay steps were done at room temperature. The assay could therefore be used in the field as part of a mass screening programme.

**References**


**Abstract**
The aim of this study was to examine the efficacy and safety of praziquantel (PZQ) in the treatment of the *Schistosoma mansoni* infection, studying the predictors of treatment failure and the performance of the IgG avidity ELISA tests in differentiating between acute and chronic schistosomiasis. All villagers living in Abis 4 village, rural Alexandria, Egypt, were subjected to stool examination, and the egg counts per gram stools (epg) were calculated. Subjects were then interviewed and examined. A single dose of PZQ was administered to all positive stool patients. Twenty four hours after treatment, all treated subjects were visited and interviewed concerning side effects. Stool analysis was performed 2 months after treatment and a second dose of PZQ was given to individuals who remained positive for *S. mansoni* infections. Cure rates and intensity reduction rates were calculated.

**Results**
PZQ is an efficacious anti-schistosomal drug as the current study revealed a cure rate of 91.1% after administration of the first dose and an overall cure rate of 98.7% after the second dose. A longer duration of water canal exposure was found to be a significant risk factor for PZQ incomplete cure (positive after first PZQ dose). PZQ was well tolerated with nausea, diarrhoea and dizziness as the only significant side-effects. Stool examination proved to be more reliable to detect cases with incomplete cure than were the studied immunological parameters.

**Conclusion**
PZQ is a safe and efficacious drug. The IgG avidity test could not distinguish between acute and chronic stages of schistosomiasis. The sharp rise of IgM in children after PZQ treatment can be attributed to their higher rates of re-infection compared to adults. Educating villagers regarding the disease and the harmful hazards of water canal exposure, as well as mass PZQ administration for children are recommended.

**Conclusions and implications of the study**
- The prevalence of *S. mansoni* in rural Alexandria accounted for 20.5%, with the highest frequency in the age group 15-30 years, but with a low grade of intensity of infection. Males recorded higher prevalence and intensity of infection compared to females.
- PZQ is an efficacious anti-schistosomal drug with an overall cure rate of 98.7% after the second dose, while the first dose left 7.6% of treated patients partially cured.
- The only significant predictor of partial cure after the first dose was the duration of water canal exposure.
- PZQ was well tolerated with mild and self-limited side-effects. Nausea, diarrhoea and dizziness were the only significant complaints.
- The IgG avidity ELISA test cannot be used to distinguish between acute and chronic stages of schistosomiasis in endemic areas because there is a continuous state of re-infection that hinders the sharp distinction between acute and chronic infections.
- The high rate of re-infection among children resulted in a sharp rise in IgM levels after treatment. Mass semi-annual PZQ administration and the organization of a health education programme to this vulnerable group are therefore recommended.

**Publications**

**Background**
Praziquantel (PZQ) is currently the drug of choice for treatment of *S. mansoni* infection as it is highly efficacious and well tolerated in a single dose, it is also widely used for morbidity control through population-based mass treatment. Variations in the cure rates between areas can be explained by differences in pre-treatment egg counts, in rapidness and intensity of re-infection. Re-infection often occurs rapidly, prepatent infections (2-5 weeks) do not respond well to treatment and may mature to productivity weeks after treatment. The cured patients may continue to excrete dead eggs for up to...
2 months which cannot be distinguished from viable eggs by the widely applied Kato-Katz method, therefore re-infection cannot be determined except after 6-12 weeks.

Recently, an assay measuring the antigen binding avidity of the IgG antibody was developed to separate low-avidity antibodies produced at an early stage of infection from those with higher avidity that reflects past infection. This test may help in the assessment of cure; low avidity was found after successful treatment and high avidity in resistance to treatment.

The aim of this study was to examine the resistance to PZQ and the risk factors for non-response to treatment. The performance of the IgG avidity ELISA test in differentiating between acute and chronic infections was also evaluated.

**Materials and methods**

A map of Abis villages in rural Alexandria was prepared and Abis 4 village was randomly selected as the study area. All households (n = 2577) were asked to provide morning stool samples, and the number of eggs per gram faeces was determined by averaging egg counts on 3 modified Kato-Katz thick smears. All subjects were interviewed using a pre-tested questionnaire. The collected information included: sociodemographic, lifestyle, history of anti-schistosomal treatment and medical past history. Clinical examination of a representative sample was carried out in the field.

**Blood samples** ELISA IgM, IgG and IgG avidity was performed on 111 consenting patients, and a complete blood count was performed to detect oesinophilia.

**PZQ treatment** A single dose of PZQ (40mg/kg body weight) was administered to all positive stool patients on December 1998 and January 1999 (non-transmission season). Pregnant and lactating women were excluded from the study. Twenty four hours after treatment, all treated subjects were visited and interviewed concerning side effects. Patients who suffered from vomiting were given a replacement therapy. Two months after treatment (February-March 1999) stools were collected and examined and epg stools were calculated. Blood samples were collected from 28 consenting patient who remained positive for *S. mansoni* to determine specific ELISA IgM, IgG antibodies and IgG avidity.

A second dose of PZQ was given to 40 individuals who remained positive for *S. mansoni* infections.

Cure rates and the intensity reduction rates were calculated. A matched control group equal in number to the cases was given a placebo to study the tolerance and safety of the drug.

**Main study findings**

*S. mansoni* is still endemic in this area with an overall prevalence of 20.5%. Prevalence increased with age to reach a plateau at 15-30 years, then was nearly stable thereafter. The intensity of infection was low according to the WHO staging system (geometric mean less than 100 epg). Males recorded higher prevalence and intensity of infection compared to females. PZQ is an efficacious anti-schistosomal drug as the current study revealed a cure rate of 91.1% after the first dose and an overall cure rate of 98.7% after the second dose. Therefore, only 7.6% of treated patients were partially cured after the first dose and the recorded response failure of PZQ was 1.3%. However, the second dose yielded 100% cure in children and an egg intensity reduction rate of 69.9% in adults who remained positive after the second dose. A longer duration of water canal exposure was found to be a significant risk factor for PZQ partial cure. This is explained by the fact that continuous exposure to water infested with cercariae allows the presence of both mature and immature forms. PZQ will kill only the mature worms, leaving the immature forms to mature and excrete eggs in stools weeks after PZQ administration.

PZQ was well tolerated with mild and self-limited side-effects. Nausea, diarrhoea and dizziness were the only significant complaints as compared to a matched control group of equal number that was given a placebo.

This study also showed that stool examination proved to be more reliable to detect cases with partial cure than immunological parameters that failed to distinguish untreated from partially cured individuals.

Therefore, the IgG avidity test cannot be used to distinguish between acute and chronic stages of schistosomiasis in endemic areas because there is a continuous state of re-infection that hinders the sharp distinction between acute and chronic infections.

There was a sharp rise of IgM in children after PZQ treatment which can be attributed to re-infection. The high rate of re-infection in children demonstrates the importance of hygienic behaviors as well as ensuring access to chemotherapy. Mass semi-annual PZQ administration to children together with a health education programme are therefore recommended.
Schistosomiasis

Ecology

Morocco

Abstract

Ecological studies on Bulinus truncatus and Planorbarius metidjensis were carried out in Agadir province, Morocco. B. truncatus was found in perennial water bodies, P. metidjensis in temporary water courses, and the species in rare habitats. Two annual generations were observed for B. truncatus, with the first from July to November and the second from November to June. P. metidjensis had 3 annual generations (May-November, October-February, and January-May). Newborn snails were exposed individually or in pairs of mixed species to miracidia of Schistosoma haematobium before being killed and examined histologically 24 hours after exposure.

Results

Miracidial penetration was greater in P. metidjensis than in the other species. Most of these larvae penetrated through the mantle in B. truncatus and P. metidjensis. Numerous live sporocysts were found in P. metidjensis whereas a rather low number was found in B. truncatus. Sporocyst migration in the snail body left sequelae consisting of tunnels. The maximum density of the sporocysts was in the foot and mantle.

Conclusion

It was hypothesized that the planorbid infection by S. haematobium occurs during the first days of snail life.

Conclusions and implications of the study

This study provided information regarding the distribution of B. truncatus and P. metidjensis in Agadir region.

B. truncatus snails were detected in perennial water bodies, P. metidjensis in temporary water courses, and the 2 species in rare habitats.

P. metidjensis has 3 annual generations. Miracidial penetration is greater in P. metidjensis than in B. truncatus. Most of the larvae penetrate through the mantle in both species. Numerous live sporocysts were found in P. metidjensis whereas a rather low number was found in B. truncatus.

The planorbid infection by S. haematobium occurs during the first days of snail life.

The results of this study provided strong and definitive evidence about the role of P. metidjensis as an intermediate host for S. haematobium.

Publications


Background

In Morocco, Agadir is the region mostly affected by schistosomiasis due to Schistosoma haematobium. Two snails were identified by the Public Health Services as intermediate hosts for the parasite: Bulinus truncatus Audouin, a classic intermediate host for S. haematobium, and Planorbarius metidjensis Forbes. This latter species was proved to be an intermediate host for the parasite in southern Portugal, but its role has never been confirmed in northern Africa and several contradicting results have been reported. A preliminary study was undertaken by the same research team in 1987 and proved the presence of the 2 snails in the region. The present study was therefore conducted in order to study the ecology of the region with special reference to the distribution of...
Schistosoma intermediate hosts, to study the biology of the snails, and to determine the role of each snail in disease transmission.

**Materials and methods**

**Studied habitats** The ecological studies were performed in the region of Agadir, where schistosomiasis cases have been detected since 1980. The water collections and irrigation canals were surveyed at regular intervals (1-3 months) to identify the species of snails in this region.

**Laboratory investigations** The physical and chemical characteristics of water samples obtained from the stations were studied. Water analysis was performed once every 2 months. Every month, 200 snails randomly selected from the 3 habitats were transported to the laboratory, 100 snails were subjected to a test of cercarial emission, while the rest was fixed and crushed for detection of the parasite in its state of evolution. Furthermore, some newborn snails were exposed to the miracidium of *S. haematobium*. A histological control was used to follow the infestation and the development of the snail in the host. The same experiment was repeated under different conditions (snail size, number of miracidia, temperature and the amount of light available for the experiment).

**Main study findings**

Eight sites of Schistosoma snails were detected in Agadir region. The ecological study revealed the following distribution of snail habitats:

1. *B. truncatus*, the classical host of *S. haematobium*, was present in the perennial water bodies. It was detected in 5 habitats: irrigation zones of Massa and Ouled Taima, rivers and the lake of Tanal, bridges of Youssef ben Tachfin and Abdel Moumen.

2. *P. metidjensis* was detected in sources and temporary water courses. It was found in 4 habitats in Assaka, Immozzer, Sidi Belkacem.

3. The 2 snails coexisted in 2 habitats: Ait Ouadrim and Ida Ougnidif.

The biology of the 2 snails was studied in Massa, Ouled Taima, Sidi Belkacem and Assaka sites Two annual generations were observed for *B. truncatus*, with the first from July to November, and the second from November to June. The life cycle of *P. metidjensis* presented 3 annual generations (May-November, October-February and January-May). These results indicate that the newborns of the schistosomiasis snail were present all year round.

It was noticed that newborn *B. truncatus* and *P. metidjensis*, when exposed individually or in pairs of mixed species to one miracidium of *S. haematobium*, miracidial penetration was greater in *P. metidjensis*. Most of these larvae penetrated through the mantle in the 2 snail species. Numerous live sporocysts were found in *P. metidjensis* whereas a rather low number was found in *B. truncatus*. The results of this study indicate that the miracidia are capable of penetrating the body of the snail and that no sporocysts can be formed during the first 24 hours. This is in disagreement with reports stating that the sporocysts were killed by the amoebicidal reaction of the snail. However, the penetration of miracidia detected in this study could be explained by the fact that newborns have immature immune systems allowing the development of sporocysts without being killed by the amoebicidal reactions. According to this hypothesis, snail infection should take place within the first few days of the snails' lives in order to reach maturation. At 24 hours, the sporocysts were detected mainly in the foot, and in the kidney and to a lesser extent in other sites. The sporocysts are in a state of continuous movement during the first days until they finally settle in the salivary glands of the snail. The other characteristics of the sporocysts (physiology, length, number of germinating cells) were less studied due to the small number of sporocysts.

**Conclusions**

This study provided information regarding the distribution of *B. truncatus* and *P. metidjensis* in the Agadir region. The detection of the 3 generations of the *P. metidjensis* all year round in this region is a finding of public health importance as the presence of newborns indicate the later development into larval stage.

Furthermore, these results provided strong and definitive evidence on the role of *P. metidjensis* as an intermediate host for *S. haematobium* in Agadir region.
Abstract

Urinary schistosomiasis transmission in Seil Al-Hasa, Jordan, was reported due to the presence of the intermediate host, Bulinus truncatus snails, favourable environmental conditions and foreign cases passing schistosomes. This work was thus undertaken to study the ecology of Seil Al-Hasa and its relation to the presence of B. truncatus along the Al-Hasa stream. Eight stations were selected to monitor the presence of snails along the Al-Hasa stream. The physical and chemical parameters of the stream water were analysed. Snails were collected from all sites, transported to the laboratory for identification and B. truncatus snails were exposed weekly to light to stimulate the shedding of cercariae for a period of 3 months.

Results

Six species of snails were collected from Seil Al-Hasa. B. truncatus snails were recorded from 2 sites in different months. They were found attached to stones in the water bed or to aquatic plants. None of the 200 collected snails shed cercariae after 3 months of laboratory observation. There was no significant difference between the sites regarding the physical and chemical parameters of the water. However, infected sites recorded higher water temperatures and lower velocities compared to the others, indicating that B. truncatus snails were able to survive by attaching themselves to stones.

Conclusion

This study emphasized the role of water temperature and velocity in schistosomiasis transmission and the need to conduct more studies on the ecology of B. truncatus.

Background

B. truncatus snails were recorded in Jordan during the period 1975-1985 from different sites: springs, irrigation pools and dams [1]. The presence of foreign workers, mainly Egyptians, infected with urinary schistosomiasis increased the probability of local transmission of the disease as the Jordanian B. truncatus was proved to be an intermediate host for Schistosoma haematobium [2]. Furthermore, autochthonous cases were also reported from Seil Al-Hasa, southern Jordan, where the Al-Hasa stream is used for land irrigation. The high temperature of the springs’ water that feeds this stream might explain the rapid proliferation of B. truncatus snails in this area in spite of Bayluscide use by the Ministry of Health. This research was thus undertaken to study the ecology of Seil Al-Hasa and its relation to the presence of B. truncatus in the stream.

Materials and methods

Eight stations were chosen as sampling points to monitor the presence of snails along the stream. The stations were visited biweekly, and snails were collected and transported to the laboratory for identification. At each visit, the following parameters were determined in each station: water temperature, dissolved oxygen, pH, electric conductivity, and water speed. In addition, bimonthly water samples were subjected to water analysis for: calcium, magnesium, sodium, potassium, chlorides, carbonates, bicarbonates, sulfates, nitrates, iron, manganese and zinc. Water analysis was performed in the Water Research Centre,
University of Jordan, according to the standard methods [3]. In addition, \textit{B. truncatus} snails collected from the sites were placed in the laboratory and were exposed weekly to light to stimulate the shedding of cercariae for a period of 3 months.

### Main study findings

Six species of snails were collected from Seil Al-Hasa. These were: \textit{Theodoxus macri}, \textit{Melanopsis praemorsa}, \textit{Melanoides tuberculata}, \textit{Gyraulus piscinarum}, \textit{Lymnaea truncatula} and \textit{Bulinus truncatus}. The latter was recorded from 2 sites during different months: from the first site during April, May, August, and September, and from the second site during May, June, and October. Hundreds of snails of this species were found attached to stones in the water bed or to aquatic plants. None of the 200 collected snails shed cercariae after 3 months of laboratory observation. There was no significant difference between the sites regarding the physical and chemical parameters of the water. However, infected sites recorded higher water temperatures and lower velocities compared to others. Water temperatures at the infected sites were 6-10 °C higher than at the other sites, and the water velocities ranged from 0.5 m/sec to 0.9 m/sec in the infected sites compared to 0.65 m/sec to 3 m/sec in the other sites.

These findings suggest that \textit{B. truncatus} snails were able to survive in the 2 sites by attaching themselves to stones, while the water velocity in the other sites was too strong such that the snails, if present, would be washed away and would not survive the water currents. These results are consistent with those obtained from a similar study conducted in Zarqa River, where \textit{B. truncatus} snails were found attached to stones in the River where a water velocity did not exceed 0.78 m/sec [4].

The identification of snails at these sites was followed by notification of the Ministry of Health, whereby snail control activities were initiated with molluscicides.

### Recommendations

The results of this study indicate the need to conduct more ecological studies, not only in endemic areas, but also in non-endemic areas, to prevent the transmission of the disease in areas with favourable environmental conditions. The study emphasized the role of water temperature and velocity in schistosomiasis transmission and endemicity and the necessity to perform in-depth analysis of these 2 factors. The disappearance of snails from the studied region for several months after snail control activities highlighted the importance of continuous monitoring of the situation and the application of molluscicides to limit the spread of the disease.

### References


Abstract
Artificial intelligence-based approaches have been used in the fields of computer science and medical diagnosis, but relatively little in epidemiological studies. This study reports the results of the application of "state-of-the-art" neural networks methodology (NNs) (a special category of artificial intelligence (AI)) to the study of schistosomiasis. A NNs structure was designed that would process and fit epidemiological data collected from 251 schoolchildren, aged 6-15 years, using first-year data to predict second-year and third-year infection rates. Data collected over 3 years included: age, sex, water canal and field exposure, medical history, and the results of stool and urine analyses. The *Schistosoma mansoni* infection rate was 50% for the baseline. Modelling was based on the standard backpropagation algorithm in which a suitable configuration of the model is built using the first year's data to optimize its performance.

Results
The performance of the NNs model in the first year compared favourably with logistic regression, but with better generalization of predicting criteria for schistosoma infection over time. The NNs model was as sensitive and specific as logistic regression, and with lower false-positive and false-negative rates. Furthermore, the relative contribution of clinical morbidity to the prediction of infection was more evident using the NNs model.

Conclusion
NNs-based models could be used as a tool in widescale control programmes, utilizing data based on unstable egg excretion and insensitive laboratory techniques, and would solve the issue of the impracticability of obtaining more than one stool or urine specimen.

Publications

Background
Neural networks (NNs) methodology is an artificial intelligence-based approach and appears to provide a highly promising computer-based methodology for pattern recognition and analysis of underlying disease dynamics. This methodology has been recently accepted by the scientific community to deal with diagnostic medical problems [1]. The objectives of this study were to design and test the feasibility of applying NNs methodology in a real-time management system of schistosomiasis control as a long-term goal, and to test the ability of this tool to predict infection under different conditions over time. The performance of such alternative non-statistical modelling was also compared with that of the conventional statistical models.

Materials and methods
Data were obtained from a previous...
study conducted in Sobtas, an Egyptian village in the Nile Delta during 1978-1980. The study population consisted of primary schoolchildren up to the sixth grade (n = 251). The collected data included: age, sex, canal water exposure, field exposure, medical history, and results of stool and urine analysis. A logistic regression model was fitted using infection as the dependent variable, with age, sex, swimming in the water canal, field work, hepatomegaly, firm liver, splenomegaly and blood in stools as independent variables. A NNs structure was designed to process and fit the collected epidemiological data using the first year's data to predict second and third year infection rates.

**Steps of neural network analysis [2]**

1. The system (i.e. a community endemic for *Schistosoma mansoni*) was observed and data collected to make a training set consisting of input variables for each individual: age, sex, swimming, etc.

2. The network representing the connections between the input and output vectors was built using the NeuroWindows computer package.

3. The training was accomplished by applying an input vector, computing the predicted output and comparing it to the observed one, and adjusting the NNs weights to minimize the difference. Each input vector is applied in turn and the network is partially trained. After a large number of applications of the input vectors, the network "converges" to a solution that minimizes the difference between the desired and measured system outputs.

4. Cross-validation was finally performed by observing how the network acted as a test data set (20% of the data).

**Performance assessment** Models were built using the first year's data. A training subset of data (80%) was used for modelling and a test subset (20%) was used for cross-validation. Both NNs and logistic models were applied to the second and third year's data of the same population to predict infection. Predictions of the 2 models were compared to the actual infection rates.

**Main study findings**

According to the statistical model, 1-year-old children were found to be 1.2 (1.06-1.39) times more likely to be infected than younger ones, and males 2.8 (1.6-4.96) times compared to females. Apart from splenomegaly, other independent variables were positively associated with infection but not statistically significant. The 2 models had comparable sensitivity and specificity, with higher sensitivity and lower specificity of the logistic regression in the last 2 years, while the NNs model had the same pattern over the 3 years with lower false-positive and false-negative rates. Furthermore, clinical morbidity had more relative contribution in the prediction of infection with the NNs model than did the logistic regression model.

This methodology is able to utilize data generated from studies about snail infectivity in water canals from different areas under different circumstances. This can be accomplished by training the NNs network to predict highly infective foci. Examples of the type of data that might be useful in building the training set for the model are: temperature, pH, depth of water, water current velocity, types and density of vegetation, snails information (density, size, infected proportion, etc.), estimated number of infective cercariae at a given water site based on cercariometry, etc. Furthermore, this methodology could be used with GIS software to characterize the environmental conditions that increase the risk of schistosoma infection, thus identifying high risk areas.

This methodology might also be applied to the epidemiological data to identify subtle population patterns associated with infection. This would increase the effectiveness of the follow-up process in assessing infection rates in the community if used in a serial screening manner with laboratory techniques. Furthermore, it might be used to study the possibility to reduce the training sample size with a reliable pattern of disease recognition, or to find the minimal subset of variables allowing accurate prediction. These approaches would reduce resources for data management and monitoring of exposed populations.

Real-time field data about the disease could also be provided based on data collected at different locations on a real-time schedule. Furthermore, the output is made to match the outcome of the real population, therefore, it could be utilized in managing the control strategies by providing a quick, accurate and low cost predictive tool.

**Conclusions**

NNs modelling showed reasonable performance when applied on population data with changing patterns over time. The potential of NNs-based models as an aid to widescale control programmes is promising.

**References**


New foci of schistosomiasis transmission in Yemen

Republic of Yemen
Maitam Valley, Ibb Governorate

Study period:
April–September 1994

Small Grants Scheme
(SGS) 1993 No. 24

Principal Investigator
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Abstract
During the period 1 April to 31 August 1994, a case-control study was conducted in Maitam Valley. The aim of this study was to investigate the health impact resulting from the contamination of the water course of the valley by the products of a malfunctioning sewerage project.

Results
The schistosomiasis prevalence was found to be 14.8% in the contaminated region compared to 4.6% in the noncontaminated upper valley. The odds ratio was 3.6 for schistosomiasis, 2.1 for trichuriasis, 1.9 for ascariasis, 1.0 for amoebiasis, 2.1 for giardiasis and 0.6 for hymenolopiasis (Hymenolepis nana). These findings confirm the adverse impact of uncompleted projects on human health.

Conclusion
The results suggest an urgent need for the chemical treatment of the water from the Ibb sewerage project, by using chlorine, insecticides and filtration to destroy the parasites, snails, various insects and active bacteria, under the supervision of the General Directorate of Public Health in Ibb Governorate.

Publications

Background
The water course in Maitam Valley runs from the southern border of Ibb city up to 20 km. The valley is characterized by good weather all year round and a temperature range of 10-30 °C. The water course flows all year and intensifies in rainy seasons (spring and summer). The population of the valley is approximately 20,000 individuals, and farming is their main occupation. Their daily activity is linked to the water canal in washing, swimming, and irrigation. In 1990, a water supply and sewerage project was established for Ibb city. The treatment plant, using the activated sludge process, was built at the head of the valley, about 5 km south of the city. The project collected different wastes that were treated mechanically, chemically and biologically. A few years after its implementation, the chemical treatment was no longer applied. As a result, untreated water was used by the villagers for domestic purposes. Furthermore, the solid disposal remaining from water separation was collected by machines and distributed to the farms to be used as untreated manure thereby creating an additional source of pollution, especially when rain washes the parasites' eggs and bacteria into the water course.

Owing to the hazards that the malfunctioning of this project has brought to the environment, the present study was conducted aiming at evaluating the contribution of the project to schistosomiasis epidemiology in the valley.

Conclusions and implications of the study
- The prevalence of Schistosoma mansoni in the lower region of the Maitam Valley, where water was contaminated by the Ibb sewerage project, was significantly higher than that of a control group living in the upper part of the valley.
- The contaminated lower region of the valley recorded significantly higher prevalence of trichuriasis, ascariasis, giardiasis and hymenolopiasis, and 80% of villagers living in the contaminated region suffered from allergic dermatitis which was mainly attributed to the bacteria used in the biological processing of the manure.
- The study recommendations were: the urgent application of chemical treatment for the water of the Ibb sewerage project, to increase the number of manure drying basins to allow for the completion of the drying process thereby decreasing environmental hazards, to educate the public regarding the disease, and to establish a health centre for the delivery of diagnostic and curative services.
Materials and methods
A case-control study was conducted in Maitam Valley, Ibb Governorate during the period 1 April to 31 August 1994. The census of the valley was conducted and the study population included villagers residing in the valley's villages. A representative sample of villagers were subjected to medical examination, laboratory investigations (stool analysis using the Kato-Katz technique, urine and blood analysis), free treatment of positive patients with praziquantel (40 mg/kg body weight), as well as free treatment of any incidental disease or emergency. Sociodemographic data were also collected during the survey.

Daily health education sessions regarding schistosomiasis and its snail intermediate host were also conducted in the villages, including measuring snail intensity in the water course. These sessions were held in mosques, weekly market gathering and other venues. Health education aids were posters and visual aids.

Main study findings
The census conducted in the present study revealed a total population of 20,334, indicating an annual increase of 3.3% in comparison with the previous 1986 census. A total of 1994 villagers were surveyed, and 1290 of them were residing in the upper part of the valley that was not affected by the project's contamination. These were taken as a control group. The identified snail intermediate host in the region was Biomphalaria pfeifferi, with an average density of 16.6%. The prevalence of S. mansoni in the contaminated region of the valley was 14.8%, with a higher frequency among males, accounting for 17.7% compared to 11.9% in females. On the other hand, the prevalence of the disease in the control region was 4.6% (8% in males and 1.4% females).

Similarly, there was a significant increase in the prevalence of other intestinal parasites in the contaminated region as compared to the control region: trichuriasis (29.2% compared to 16.2%), ascariasis (56.1% compared to 40.3%) and giardiasis (23.4% compared to 12.4%). However, there was no significant difference between the 2 regions regarding the prevalence of amebiasis (67% compared to 66%). Taenia species also recorded a significant increase among residents of the contaminated region (9.3% versus 6.3%). Interestingly, 80% of villagers living in the contaminated region reported variable grades of allergic dermatitis up to skin ulceration, a condition which was not seen in the control group. This dermatitis was mainly attributed to the bacteria used in the biological processing of the manure.

Conclusions and recommendations
It was concluded that urgent chemical treatment for the water of the Ibb sewerage project was mandatory in order to kill active bacteria, helminths and parasite eggs. It was recommended to increase the number of manure drying basins to at least 6, because the 2 available basins did not allow for the completion of the drying process, thereby increasing environmental hazards. It was also recommended to communicate the hazards of water canal exposure to the villagers through continuous health education sessions. Finally, this study emphasized the need to establish a health centre for the delivery of diagnostic and curative services.
Schistosomiasis among schoolchildren in Al-Mahweet Governorate, north-west Yemen

Republic of Yemen
Al-Mahweet Governorate, North-west Yemen

Study period:
December 1996–1997

Small Grants Scheme
(SGS) 1996 No. 6

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Prevalence rates of helminthic infections among schoolchildren were: 22% for *Schistosoma mansoni* infection, 7% for *Schistosoma haematobium* infection, 61% for ascariasis, 21% for trichuriasis, 2% for fascioliasis, 0.3% for enterobiasis, 0.7% for hook worm infection and 0.2% for strongyloidiasis. Availability and use of latrines were two factors implicated in the transmission of soil-transmitted helminths. Use of latrines, sex, water contact and age were the determinants of *Schistosoma* infection.

There was significant lowering in the prevalence and intensity of infection after treatment in *S. mansoni*, *A. lumbricoides*, and *T. trichiura*, but not in *S. haematobium* or other soil-transmitted helminths.

Mass chemotherapy, health education and snail control measures were the main treatment recommendations.

## Abstract

The prevalence, intensity and incidence of schistosomiasis and soil-transmitted helminthiases among schoolchildren in an ignored area in Yemen were determined. The study aimed to investigate the impact of single doses of praziquantel or albendazole or both, and how they relate to sanitary, socioeconomic and behavioural practices on the prevalence and intensity of infections. Out of a total number of 897 pupils, 453 were randomly selected from Al-Mahweet town and 444 from rural surrounding areas. Millipore filtration, modified Kato-Katz and precipitation techniques were applied for urine and stool analysis.

**Results** Prevalence rates were 28% for schistosomiasis, 61% for ascariasis, 21% for trichuriasis, 2% for fascioliasis, 0.3% for enterobiasis, 0.7% for hook worm infection and 0.2% for strongyloidiasis. Factors found confounding the relationship between schistosomiasis and residence, under logistic regression analysis, were sex and frequency of water contact. The probability of infection with schistosomiasis for boys who reside in rural Al-Mahweet and visit the water source is 0.52, compared to 0.30 for those who reside in Al-Mahweet town. Odds ratio estimates accounted for were 2.5 for residence, 1.7 for water contact and 3.2 for boys. With regards to other helminthic infections, availability of latrines remained the only significant determinant of infection. Treatment reduced the infection rate of *Schistosoma mansoni* by 62.5%, *Trichuris trichiura* by 48% and *Ascaris lumbricoides* by 24%.

**Conclusion** Annual campaigns of treatment can reduce the burden of *Schistosoma mansoni*, trichuriasis and ascariasis. On the other hand, for *Schistosoma haematobium*, the appropriate time interval for intervention should be shortened according to the findings of a properly designed intervention study before being used as a single control measure. Since 77% of the children were infected with other helminths, mass treatment should be extended to cover all children. For those boys in rural Al-Mahweet who are continuously exposed to the water source, mass treatment for schistosomiasis was recommended since the prediction of infection rate reached 52%.

**Publications**

**Training**
A mobile team of researchers and technicians was trained on the methods of conducting epidemiological and field surveys.

**Background** Both *Schistosoma haematobium* and *S. mansoni* are endemic in Yemen, with a prevalence ranging from 20%-40%. However, there is a lack of information regarding the disease in some regions of the country such as Al-Mahweet Governorate. This study was conducted in order to estimate the prevalence of schistosomiasis and other soil-transmitted helminthiases in schoolchildren in Al-Mahweet Governorate.

**Materials and methods**
Al-Mahweet city, 113 km from Sana’a,
lies about 2000 m above sea level, with extensive agriculture that depends on rains and groundwater. There are about 430 schools in the entire governorate with a total of 57,000 students enrolled. A total of 498 students (186 girls and 312 boys) were randomly selected from the mixed primary schools in Al-Mahweet town. Four hundred and fifty-three additional students were randomly selected from the rural areas of Al-Mahweet Governorate. Therefore, a total of 951 children were enrolled in the study.

Detailed personal and behavioural information were collected. Midday urine and stool samples were obtained from each individual and examined using the Millipore filtration technique and the modified Kato-Katz technique for urine and stool analysis, respectively. Other ova, cysts or larvae were also recorded. Single doses of praziquantel and/or albendazole were administered whenever an infection was confirmed.

Follow-up was conducted one year later by urine and stool collection. Children were re-examined and those with a positive infection were treated again, while other children with soil-transmitted helminthiasis were referred to the hospital.

Main study findings
A total of 948 students participated in the study and fulfilled a complete set of investigations. More than half of these students (51%) were living in urban areas and the remaining children were from the surrounding villages. Almost half of the children had access to latrines, but there was a significant difference between those living in urban and rural areas regarding the use of latrines, with 78.3% using latrines in urban compared to 17.4% for those living in rural areas.

Prevalence rates were 22% for Schistosoma mansoni infection, 7% for S. haematobium infection, 61% for ascariasis, 21% for trichuriasis, 2% for fascioliasis, 0.3% for enterobiasis, 0.7% for hook worm infection and 0.2% for strongyloidiasis. Factors found confounding the relationship between schistosomiasis and residence, under logistic regression analysis, were sex and frequency of water contact. The probability of infection with schistosomiasis for boys who reside in rural Al-Mahweet and visit the water source is 0.52, compared to 0.30 for those who reside in Al-Mahweet town. Odds ratio estimates accounted for via residence was 2.5, via water contact 1.7 and via boys 3.2. With regard to other helminthic infections, the availability of latrines remained the only significant determinant of infection.

There was significant lowering in the intensity of infection (geometric mean) after treatment of cases of S. mansoni infection but no difference in cases of S. haematobium infection. Similarly, the prevalence of infection was significantly reduced in cases of S. mansoni, A. lumbricoides, and T. trichiura. On the other hand, there was no significant lowering in the prevalence of S. haematobium infections or other soil-transmitted helminthiasis after treatment.

Conclusions and recommendations
This study revealed that helminthic infections were affecting a considerable proportion of schoolchildren in this area. Annual campaigns for examination and treatment were recommended in order to reduce the prevalence and morbidity of schistosomiasis in this population. A mass chemotherapy campaign for the other helminths could be beneficial in the control of these infections.

The availability and use of latrines were two factors implicated in the transmission of soil-transmitted helminthiasis. Use of latrines, sex, water contact and age groups were the determinants of Schistosoma infection.

Health education of the community to encourage the construction and use of latrines would produce a significant impact on the control of helminthic infections. It was finally recommended to conduct surveys on the snail population in the different water bodies and to plan a snail control strategy for the area.
Abstract
The introduction of irrigation schemes to the Sinai through the El-Salaam canal to reclaim new areas is an ongoing project. Although these irrigation channels provide substantial benefits in the form of employment opportunities and decreased dependence on food imports, it may create a new environment allowing for the transmission of schistosomiasis to the local population. The current study was therefore conducted to determine the prevalence and risk factors of schistosomiasis in both bedouin and immigrant populations and to evaluate their knowledge, beliefs and behaviour regarding the disease. Moreover, a follow-up study was conducted to evaluate the response 6 months after praziquantel treatment of infected persons.

Results
The overall prevalence of schistosomiasis was 6.2%. It varied among different groups and areas: it was 5% in bedouins, 7.6% in immigrants, 10% in relatively old reclaimed areas, and 5.1% in recently reclaimed areas. Immigration from the Nile Valley, residency in relatively old reclaimed areas, male gender and illiteracy were the main risk factors for schistosomiasis in the Sinai. There were significant differences in knowledge and beliefs between bedouins and immigrants. The behaviour in both groups towards the disease was nearly similar. Furthermore, there was no significant difference between both groups regarding the 6-month response to single praziquantel administration as 80% of cases remained negative, indicating a slow rate of reinfection in the area.

Conclusion
The present study emphasized the need to optimize future strategies in order to prevent the establishment of schistosomiasis in the newly reclaimed areas.

Background
Emerging infectious diseases can be defined as infections that have newly appeared in a population or are threatening to increase in the near future. Specific factors precipitating disease emergence can be identified including ecological, technological, biological or demographic factors that place people at increased risk of contracting infection. Therefore, in the case of a dramatic change in the environment, such as that which accompanies new irrigation projects, there is a need for effective surveillance and control of emerging diseases. In Egypt, 2 major irrigation schemes for land reclamation have been recently implemented: the El-Salaam irrigation project in the northern Sinai peninsula, and the Toshka project in southern Egypt. This study was conducted to determine the prevalence of schistosomiasis in the newly reclaimed areas of the northern Sinai peninsula, to identify the risk factors of schistosomiasis infection, and to evaluate the knowledge, beliefs and
behaviour of the studied communities and their response to a single dose of praziquantel, 6 months after treatment.

**Materials and methods**

The study population included all individuals aged 6-60 years. Data on knowledge and beliefs related to water contact and schistosomiasis were collected using a semi-structured questionnaire from the family heads and health workers, while the behaviour and practice of the inhabitants were observed. A cross-sectional survey of the study area was undertaken according to a list of a representative sample of households. The collected information included demographic data and place of origin. All inhabitants included in the survey were subjected to: clinical examination for schistosomiasis or its complications, stool examination, urine analysis, abdominal ultrasonography, and serological investigation by indirect haemagglutination test.

The evaluation of the knowledge, beliefs and behaviour was performed by interviewing a representative sample of bedouins (530 individuals) and immigrants (450 individuals).

A nested case-control study design was used to study the risk factors of schistosomiasis among infected cases as compared to non-infected controls.

All positively diagnosed individuals treated with praziquantel (40 mg/kg body weight) during the initial cross-sectional survey were evaluated 6 months later in a follow-up study.

**Main study findings**

The prevalence of *Schistosoma mansoni* in the newly reclaimed areas in northern Sinai was 6.2%, and no cases of *S. haematobium* were diagnosed. There was a significantly higher prevalence of schistosomiasis in areas with established agriculture compared to areas with recently implemented agricultural activities related to water contact. By contrast to infection in the Nile Valley, the prevalence of infection was higher in older individuals; this could be explained by the fact that most infections were acquired during visits to the Nile Valley, which is mostly an adult activity. Male sex and the farming occupation were the significant risk factors for the disease mainly due to the greater liability of these groups to exposure to water canal. Measures of socioeconomic status such as educational level were also implicated with contracting infection. Furthermore, immigrants were at a significantly higher risk for schistosomiasis compared to bedouins.

There was a significant difference between immigrants and bedouins regarding the knowledge and beliefs of schistosomiasis. The reaction to health education and its interpretation, as well as the behaviour towards the disease were comparable in the 2 groups.

After 6 months of praziquantel administration, 80% of infected bedouins and immigrants remained negative, indicating that the reinfection rate was relatively limited during a 6-month period.

**Conclusions and recommendations**

The emergence of schistosomiasis in the newly reclaimed land in North Sinai highlighted the need for active surveillance along the El-Salaam canal in order to prevent the establishment of the disease in this area. An integrated approach consisting of chemotherapy and targeted health education was recommended in order to limit reinfection and to control the disease.
Abstract
This study aimed at determining the current status of schistosomiasis infections in the Gezira-Managil scheme and the impact of a new irrigation system on transmission. Schoolchildren from 15 randomly selected schools were subjected to parasitological examination of stool and urine. All children ≥10 years old were interviewed regarding risk factors of disease transmission, and a sample of children was clinically examined. The transmission of schistosomiasis was studied in Agando village, Gezira area and in 2 camps, by parasitological survey of the residents as well as by studying the effect of drying and cleaning canals on snail density and infection with schistosomes.

Results
The prevalence of infection among 3170 schoolchildren was 15.8% and 1.8% for Schistosoma mansoni and S. haematobium, respectively, being significantly higher in the Managil than in the Gezira area, with no significant difference between the 2 areas regarding the intensity of infection. The significant risk factors in the 1648 interviewed children were: farming as the paternal occupation, living in houses built with material other than red bricks, proximity to a water canal, unavailability of water supply and latrines, and past history of infection and treatment. In the camps, the prevalence of S. mansoni infection was significantly higher than that in the villages. The drying and cleaning of canals reduced the snail density and the snail intermediate hosts.

Conclusion
S. mansoni infection is the predominant type of schistosomiasis in the area. An integrated control programme particularly targeting the camps in the neighbourhood of the villages was highly recommended.

Background
The prevalence of S. mansoni in the Gezira-Managil scheme is high while that of S. haematobium is generally low and variable from one village to another. A comprehensive control strategy consisting of chemotherapy, focused mollusciciding, health education and improvement of water supply and sanitation was adopted and implemented by the Blue Nile Health Project (BNHP) during the period 1980-1990. In April 1999, the Gezira Administration Board adopted a new system for improving irrigation, consisting of drying and cleaning canals before the irrigation season. This was expected to lower the snail density in the area. The tendency of snails to colonize the tails of canals, where stagnant water enhances the growth of vegetation, indicates the importance of proper maintenance of new irrigation systems.
Materials and methods

The prevalence of schistosomiasis was studied in 15 randomly selected schools from the 45 schools in the area where the Blue Nile Health Project was implemented. Parasitological examination of stool and urine samples was performed. Stool specimens were examined using the locally modified Kato-Katz method and urine specimens were examined using the simple sedimentation technique.

All children ≥10 years old were interviewed using a questionnaire. The information collected was sociodemographic data, housing conditions such as water supply and sanitation, water contact activities, knowledge about schistosomiasis, and history of schistosomiasis infection and treatment. Children from 4 schools (2 from each area) were subjected to clinical examination.

The transmission of schistosomiasis was studied in Agando village, Gezira area, which includes the village and 2 labour camps, as well as 2 neighbouring labour camps with residents coming from western Sudan. These camps are highly dependent on the water canal and lack services such as water supply, health services, and schools.

A census of the 2 camps was performed and stool and urine samples were collected and examined from all the residents.

The effect of drying and cleaning the canals from vegetation and silt accumulation on the snail population and transmission of schistosomiasis was studied in the canals around Angado village in the Gezira area. This activity was performed before the irrigation season, and snails were collected monthly from different sites at the head, middle, and tails of minor canals. Snails were also collected from human water contact sites in the village area and from the neighbouring camps in order to determine the seasonal changes in snail densities and infection with schistosomes. Snails were collected using scoops (10 scoops from each site), and subsequently counted and sorted by species in a field laboratory. *Biomphalaria pfeifferi* and *Bulinus truncatus* were examined for schistosome infection.

Infected schoolchildren and camp residents were treated with praziquantel (40mg/kg body weight).

Main study findings

A total of 3170 schoolchildren were subjected to stool and urine examination. The prevalence and intensity of infection were 15.8% and 112.7 eggs/g for *S. mansoni*, compared to a prevalence and intensity of 1.8% and 7.3 eggs/10 ml, respectively, for *S. haematobium*. The prevalence of both species was significantly higher in the Managil area than in Gezira, compared to 2.6% and 0%, respectively, for *S. haematobium*.

Regarding the clinical examination, there was no significant association between the results of parasitological examination and certain signs and symptoms such as abdominal pain, blood in the stool, diarrhoea and fever. On the other hand, there was a significant association with visible haematuria and splenomegaly. No hepatomegaly was reported among the schoolchildren examined.

A total of 1648 children were interviewed regarding the risk factors for schistosomal infection. The most significant identified risk factors were: farming as a paternal occupation, poor housing conditions such as living in houses built with material other than red bricks such as mud or straw, and proximity to the water canal. The risk of infection was significantly associated with the unavailability of water supply and latrines. House tap water was reported to exist for 97% and 56% of schoolchildren interviewed in Gezira and Managil, respectively. Furthermore, past history of infection and treatment was a significant risk factor for infection. Half the interviewed children did not know about the hazards of water canal contact, and 27.2% did not have any information about the mode of transmission of the disease, however, knowledge and awareness about the disease did not have a positive impact on disease transmission.

The prevalence of infection in the labour camps was significantly higher than that in the villages, amounting to 39.9% and 0.8% for intestinal and urinary schistosomiasis, respectively. The prevalence of infection was higher in males than in females, but this was not statistically significant. The age-specific prevalence was 45.4% among children younger than 10-years old, reached a peak in the age group 10-19 years (67.3%), then declined thereafter.

The effect of drying and cleaning canals was reflected in the snail density, which decreased, particularly in the Gad Elain minor canal. The snail intermediate hosts were absent around Angado village, but were found in low densities in the camps’ (Gad Elain, Toba and Elku) minor canals, mainly in the tail parts.

Conclusions and recommendations

*S. mansoni* infection is the predominant type of schistosomiasis in the area. The lower prevalence of infection in the Gezira compared to the Managil area highlights the long-lasting impact of the Blue Nile Health Project for schistosomiasis control. Provision of a safe water supply, better housing conditions, and maintenance of the new irrigation system in the scheme were the main study recommendations. There was also a major emphasis on the need to implement a control programme targeting the camps in the neighbourhood of the villages.
Abstract
The long-term effect of a single dose of praziquantel on morbidity and mortality from Schistosoma mansoni was investigated in surveys in 1987 and 1994 in central Sudan. Prevalence of infection dropped from 53% to 34%, and intensity of infection (> or = 400 eggs/g of faeces) from 31% to 18%. There was a reduction in hepatomegaly and hepatosplenomegaly, although splenomegaly alone was unchanged. Prevalence of periportal fibrosis decreased from 14% to 10%. Endoscopic investigation of patients with fibrosis showed a reduction in oesophageal varices from 47% to 30%. Mortality due to bleeding varices was high (community-wide, up to 11/100 infected patients with bleeding). Thus praziquantel mass treatment can be spaced to a much longer period, reducing the expense of treatment, delivery and distribution.

Publications
Kheir MM et al. Effect of single-dose praziquantel on morbidity and mortality resulting from intestinal schistosomiasis. EMHJ, 2000, 6:926-931.

Background
Although much research has been performed in the epidemiology, treatment and immunology of schistosomiasis, there is a paucity of information regarding the morbidity and mortality of this disease. Many of the studies were hospital based and limited in the selection of patients. In a survey conducted during the period 1985-1987, the prevalence of Symmer's fibrosis in the population was 13.6%, of whom 54% reported oesophageal varices [1]. In another retrospective study, annual treatment of villagers produced regression in the signs of mild fibrosis and protected against the occurrence of periportal fibrosis [2]. However, the protective effect of a single dose of praziquantel (PZQ) was not studied, nor the frequency of its administration required to produce such a protective effect. The objectives of this study were: to examine the effect of single praziquantel therapy in the long-term reduction of hepatosplenomegaly and Symmer's periportal fibrosis by comparing the results of the current survey, conducted in 1994, with those of an initial survey conducted in 1987 in the same setting and using the same methodology; to evaluate the natural history of fibrosis and/or oesophageal varices in patients treated with praziquantel, 7 years earlier, during the initial 1987 survey.

Materials and methods
The records of the earlier studies conducted in the eighties were reviewed in order to identify the villages previously surveyed and Abu Jin village, Gezira state, was selected as the study area. The village is located between the 2 Nile rivers in Sudan, 150 km south-west of Khartoum. A complete census of the village was performed followed by tracing the causes of deaths through the local dispensaries and El-Ribie Hospital (local district hospital). The prevalence of infection and its intensity were determined by stool examination using the Kato-Katz method. A random sample of 240 villagers was interviewed for the following: history of intestinal and urinary schistosomiasis, jaundice, haematemeses, past history of haematemeses, and history of antischistosomal treatment. The villagers were also subjected to clinical examination of the liver, spleen, ascites, anaemia, and signs of portal hypertension.

Conclusions and implications of the study
- A single-dose of praziquantel had a significant impact on the disease prevalence, intensity of infection, burden of chronic liver disease and mortality due to schistosomiasis mansoni.
- Mass treatment can be spaced to a much longer period, reducing the expenses of treatment, delivery and distribution.
The villagers were finally referred for ultrasound examination, and those with Symmer’s fibrosis were re-assessed and carefully questioned about previous attacks of haematemesis and loss of blood. In 1987, 32 patients were randomly selected among those with Symmer’s fibrosis, and their degree of periportal fibrosis (PPF) was recorded. They were subjected to endoscopic examination for evidence of oesophageal varices, and the degree of varices was graded. The follow-up of this cohort of patients in 1994 revealed that 2 patients had died from haematemesis, and the remaining 30 patients accepted participation in the study. None of them had a history of haematemesis, jaundice or abdominal distension. They were subjected to: stool analysis, clinical examination, ultrasonography and endoscopy.

Main study findings

During the period 1987-1994, the village population was rather stable with only few immigrants or new settlers (1190 compared to 1080 villagers in 1994 and 1987 censuses, respectively). There was no change in the social habits or farming-affecting water contact, nor in the sanitary system; no additional pit-latrines or tap water supplies were introduced apart from the old common tap. The prevalence and intensity of infection were significantly reduced in 1994 compared to the initial study in 1987 by the single dose of praziquantel administered 7 years earlier; the prevalence of schistosomiasis dropped from 52.5% to 33.9%, the frequency of the peak intensity in 10-19 year-olds was also reduced from 73.3% to 51%, and the frequency of egg counts >400/g was reduced from 31% to 17.7%. On the other hand, there was no significant difference between the 2 surveys regarding the frequency of organomegaly (hepatomegaly and/or splenomegaly).

Considering the cohort of 32 patients previously diagnosed and treated in 1987, the single dose of praziquantel administered in 1987 resulted in a significant drop in the frequency of infection, egg load and intensity of infection, and a significant reduction in the frequency of organomegaly, except splenomegaly, which could be attributed to concurrent infections, e.g. malaria. Interestingly, a single dose of praziquantel resulted in the complete regression of fibrosis in 3 patients with grade 1 PPF, and 11 patients remained grade 1 PPF over this 7-year period without developing haematemesis or melena. Similarly, regarding the endoscopy results, patients regressed to a less severe grade of oesophageal varices during that period. The overall frequency of oesophageal varices in patients previously diagnosed as having Symmer’s fibrosis in 1987 was significantly reduced from 47% to 30% over these 7 years.

Conclusions and recommendations

In spite of frequent schistosomical reinfection, a single dose of praziquantel administration resulted in a significant decrease in the prevalence of infection and the regression of early signs of schistosomal morbidity. The results of this study also proved the effectiveness of administering the drug at wider intervals (up to 7 years), instead of the usual annual or biannual praziquantel administration, which could be a cost-effective approach for schistosomal control in communities with limited resources.

References

Abstract

Hepatosplenic schistosomiasis and human fascioliasis coexist in the Abis area of rural Alexandria. This study was conducted in order to assess morbidity in patients with single or combined infections. Stool samples were collected twice from 3658 villagers in Abis I village and examined by the Kato-Katz technique. A random sample of positive cases (n = 281) was subjected to a detailed morbidity study. All cases were hospitalized and subjected to clinical examination, ultrasonography, liver function tests and tests for connective tissue markers. A liver biopsy was performed in 37 patients. Results were compared with healthy controls matched for sex and socioeconomic status.

Results

Single and combined infections with Schistosoma mansoni affected 29.5% of the rural population (21.5% schistosomiasis and 8% fascioliasis). S. mansoni infection was more prevalent among young adults (15-35 years of age), while fascioliasis and mixed infections were more prevalent among children. Males were more infected with schistosomiasis and mixed infections compared to females, while females were more infected with fascioliasis. The majority of affected cases, whether in schistosomiasis or fascioliasis, recorded low intensity of infection. The highest procollagen III peptide levels were observed in cases with mixed infections. The serum fibronectin level was lower in cases compared to controls, and in schistosomiasis cases compared to Fasciola cases. Younger age was a significant predictor for elevated procollagen level. In cases with schistosomiasis, serum fibronectin was positively correlated with serum albumin, and procollagen III peptide was positively correlated with serum alkaline phosphatase (SAP). Egg count was directly correlated with SAP in Fasciola cases. In mixed infections, the Schistosoma egg count was positively correlated with SGPT, serum fibronectin was directly correlated with prothrombin activity, and procollagen III peptide with SAP.

Conclusion

Single and combined infections with S. mansoni and Fasciola proved to be a relevant public health problem in Abis I village, as 29.5% of the surveyed population had either or both infections.

Conclusions and implications of the study

- Single or combined infections with Schistosoma mansoni (21.5%) and Fasciola (8%) proved to be a relevant public health problem in Abis I village, as 29.5% of the surveyed population had either or both infections.
- Procollagen III peptide levels were elevated in cases with mixed infections, followed by Schistosoma mansoni, indicating that mixed infection has stimulated fibroblastic proliferation and procollagen synthesis.
- The serum fibronectin level was lowest in schistosomiasis cases, intermediate in mixed, and highest in Fasciola cases, indicating more fibrogenesis induced by Schistosoma infection.
- Younger age is a significant predictor for elevated procollagen level due to age-related variability in fibroblastic stimulation triggered by these parasitic infections.
- Connective tissue markers can identify active ongoing fibrosis, while ultrasonography refers to old ones. Procollagen III peptide could be used for early diagnosis and management, and therefore regression of the signs of fibrosis. The survey emphasized the importance of prophylactic strategies directed to children living in this area.

Publications

Abu Basha et al. Hepatic fibrosis due to fascioliasis and/or schistosomiasis in Abis village, Egypt. EMHJ, 2000, 6:870-878.

Background

Hepatosplenic schistosomiasis is the most important form of morbidity caused by S. mansoni. Human fascioliasis is another additional hazard to the liver. These 2 conditions coexist in the Abis area, rural Alexandria, Egypt. The purpose of this study was to assess morbidity in patients with schistosomiasis, fascioliasis and combined infections.

Materials and methods

The selection of Abis I village was...
based on a previous survey that confirmed the endemicity of schistosomiasis and fascioliasis in this village. A map was prepared and the houses, totalling 589, were enumerated. Random selection of 403 houses was performed. Stool samples were collected twice from 3658 villagers and examined by the Kato-Katz technique. A random sample of positive cases (n = 281) was subjected to detailed morbidity study. Exclusion criteria were: history of jaundice, viral hepatitis, history of intake of anti-rheumatics, analgesics, drugs for schistosomiasis or fascioliasis during the last 6 months.

All cases were hospitalized and subjected to clinical examination, ultrasonography, and blood samples were collected for complete blood picture, liver function tests, hepatitis B surface antigen, connective tissue markers, fibronectin and procollagen III peptide. Liver biopsy was performed in 37 patients (14 with schistosomiasis, 14 with fascioliasis, and 9 with mixed infections). Results were compared with healthy controls matched for sex and socioeconomic status as cases.

Main study findings

The prevalence of *S. mansoni* was 21.5% (19% single infection and 2.5% mixed infection with Fasciola). The prevalence of Fasciola infection was 8% (5.5% single and 2.5% mixed with *S. mansoni*). *S. mansoni* infection was more prevalent among the age group 15-35 years (25.4%), followed by children (19.6%), while fascioliasis and mixed infections were more prevalent among children (9% and 4%, respectively), followed by adults (4% in both). Males were more infected than females with schistosomiasis (30.6% versus 13.4%), while females were more infected with fascioliasis (10% versus 7%). Mixed infections were more frequently encountered in males (2.8%) compared to females (2.3%). Regarding the intensity of infection, 83%, 15% and 2% suffered from low, moderate and heavy intensity of Schistosoma infection, respectively. In fascioliasis, 91.3% suffered from low intensity of infection compared to 8% and 0.7% of moderate and heavy intensity of infection, respectively.

Detailed morbidity study

The highest procollagen III peptide levels were observed in cases with mixed infections, followed by *S. mansoni*, indicating that mixed infection has stimulated fibroblastic proliferation and procollagen synthesis. The serum fibronectin level was lower in cases compared to controls, and in schistosomiasis cases compared to Fasciola cases, indicating that the fibrogenesis induced by Schistosoma infection is more than that induced by Fasciola. In mixed infection, the serum fibronectin level was of intermediate level, which could be attributed to the immunoregulatory mechanism guarding against dramatic decrease in serum fibronectin level in concurrent infection. The decrease in fibronectin level in liver diseases was attributed to disorders in coagulation.

On the other hand, procollagen peptide III level was increased in cases, and was significantly higher in children compared to adults. After adjusting for confounding variables, age remained a significant predictor for elevated procollagen level. This could be explained by age-related variability in fibroblastic stimulation triggered by such parasitic infections. There was no association between connective tissue matrix markers and the grade of liver fibrosis diagnosed by ultrasonography. This was explained by the fact that the tissue markers can identify active ongoing fibrosis, while ultrasonography refers to old ones.

There were significantly higher levels of SGOT and serum alkaline phosphatase in patients affected with single or mixed parasitic infections compared to controls. In cases with schistosomiasis, serum fibronectin was positively correlated with serum albumin such as the situation in alcoholic cirrhosis. Furthermore, procollagen III peptide was positively correlated with SAP, indicating active hepatic fibrosis.

In cases with fascioliasis, the egg count was directly correlated with SAP, which could be explained by the fact that the number of eggs reflect the adult Fasciola worms that may lead to biliary stasis and obstruction.

In cases with mixed infection, the egg count was positively correlated with SGOT and SGPT for Schistosoma eggs but not for Fasciola’s. In this group of individuals, the decrease in serum fibronectin was directly correlated with a corresponding decrease in prothrombin activity, and procollagen III peptide was also correlated with SAP.

Conclusions and recommendations

Single and combined infections with *S. mansoni* and Fasciola proved to be a relevant public health problem in rural Alexandria, Egypt. The survey highlighted the importance of prophylactic strategies directed to children living in this area. The increase of procollagen III peptide indicated active fibrosis, and it was therefore recommended to use this connective tissue marker for early diagnosis and management, and therefore the regression of the signs of fibrosis.
Abstract
This study was conducted to estimate the impact of schistosomiasis on the socioeconomic level and on the quality of life (QOL) and productivity of workers. All workers in Mısır Textile company, Kafr-El-Dawar, Beheira Governorate, suffering from active or chronic schistosomiasis were included in the study. An equal number of healthy controls matched for job similarities to the cases were also included. Data collection forms included information regarding sociodemographic, occupation, housing conditions, clinical data, and knowledge of and attitudes towards schistosomal infection. The WHOQOL instrument for measuring quality of life was used. Data were also collected from the medical records of workers in the outpatient and inpatient settings as well as from the personal records of the workers available in the company. The latter included information regarding workers' productivity, sickness, injury, and days lost. Disability data were obtained from the social insurance department and cross-checked with the labour office of the town. Schistosomiasis stages ranged from active infection to urinary cancer for Schistosoma haematobium or haematemesis for S. mansoni.

Results
Most patients suffered from S. mansoni, the most frequent symptoms being dysuria, frequent micturition and dysentery. Hepatomegaly and splenomegaly were detected in 29% and 39%, respectively. Few patients had ascites, ankle oedema or haematemesis. A minority reported bladder ulcers, ureteric strictures, cor pulmonale or heart failure. Patients had significantly better knowledge, higher rates of utilization of medical services, and greater economic burden and disability rates compared to the controls. Furthermore, the mean productivity score of patients was lower than that of controls. Patients recorded significantly lower levels of quality of life, and this was strongly associated with the stage of the disease. QOL domains, additional working hours, total incentives, and the total cost of illness could be used as discriminators for the schistosomiasis.

Conclusion
Schistosomiasis has a negative impact on the quality of life and productivity of the affected individuals.

Publications

Activities achieved within the framework of the study
Training of data collectors and field supervisors included discussion of the objectives and importance of the study, orientation about the questionnaire, method of data collection and editing. The practical training of company physicians was carried out in the company hospital.

Background
An effective disease intervention is one that produces a net improvement in the beneficiaries' quality of life and/or increases life expectancy. Unlike developed countries, there is a scarcity of information on the measures of the ultimate output of health care in developing countries. Quality of life (QOL) is measured as physical and social functioning and as perceived physical and mental well-being. Health-related quality of life (HRQL) includes aspects of physical, psychological, and social well-being issues for people.
Although schistosomiasis is endemic in many tropical regions of the world, studies have only dealt with the diagnostic and clinical aspects of the disease. Most of the studies dealing with the impact of schistosomiasis used mortality and morbidity as the main indicators. However, morbidity studies seldom included the impact of the disease on the physical and socioeconomic development of an affected individual and on the economic and social consequences affecting community development. Therefore, this study was conducted to estimate the impact of schistosomiasis on the socioeconomic level as well as on the quality of life and productivity of workers.

Materials and methods
All workers in Misr Textile company, Kafr-El-Dawar, Beheira Governorate, suffering from active or chronic schistosomiasis were included in the study. An equal number of healthy controls matched for job equivalence to the patients were also included.

Study tools
Four types of questionnaire were used for data collection. The first included information regarding sociodemographic, occupational and home environment, and clinical data of the disease. Data related to knowledge and attitudes towards schistosomal infection were also included in this first questionnaire. The WHOQOL instrument for measuring QOL was used in a second questionnaire. The instrument consists of 26 questions, each of five-point scale. The scale consists of five domains: domain 1 is concerned with the physical and independence quality, domain 2 with the psychological and spiritual aspect, domain 3 with the social aspects, and domain 4 with the quality of the environment.

A third form was designed to collect data from the medical records of workers in the outpatient and inpatient settings. A fourth form compiled data from the workers’ records in the company such as the measures of productivity (e.g. production scores, penalties, incentives, discipline and salary), as well as data related to sickness, injury and days lost. Disability data were obtained from the social insurance department and cross-checked with the labour office.

Data collection was performed by staff members of the Community Medicine Department, Faculty of Medicine and Faculty of Nursing as well as the company physicians.

QOL score
The score of each domain was calculated so that the positive and negative questions would add up in the same direction. The total score was then transformed onto 0-100 score.

Production score
According to the company criteria, each worker was given a score between 0 and 100 each month. The total score was calculated by addition of the monthly scores. Absence frequency, severity rates and proportional sickness disability rates were also calculated.

Scoring of schistosomiasis stages
This score ranged from active infection (score 0) to urinary cancer from S. haematobium or haematemesis from S. mansoni (score 5).

Calculation of cost
The receipts of hospitals, investigations performed, drugs prescribed and any medical procedure were included in the calculation of costs.

Main study findings
Schistosomiasis workers and controls were comparable regarding age, gender, job category and almost all were married. The control group and their spouses had significantly higher educational levels, tended to live in urban areas, recorded a higher mean crowding index and income, and also lived in better housing conditions (including sewage and refuse disposal, electricity, etc.) compared to the patients.

Most of the patients (78.8%) suffered from S. mansoni and only 7.6% from S. haematobium, while the rest suffered from mixed infections. The most frequent symptoms were dysuria, frequent micturition, and dysentery. Hepatomegaly and splenomegaly were detected in 29% and 39% of infected workers, respectively. Few patients had ascites, ankle oedema or haematemesis. A minority reported bladder ulcers, ureteric strictures, cor pulmonale or heart failure.

Patients had significantly better knowledge concerning all aspects of the disease. They also recorded higher rates of utilization of medical services, and their mean costs for these services was significantly higher than for the controls. Disability rates were also higher among patients, but there was no significant difference between both groups regarding sickness absence rates, and the mean productivity score of patients was lower than that for controls.

Regarding QOL, sufferers recorded significantly lower general domain, physical, independence, psychological, spiritual and social domains, but the environmental domain was lower in the control group. This was strongly associated with the stage of the disease; a better QOL was always associated with earlier stages of the disease. QOL domains, additional working hours, total incentives, and total cost of illness could be used as discriminators for the disease, as revealed by multiple logistic regression analysis.

Recommendations
The results of this study highlighted the need for planning and implementation of a health education programme for workers. Pre-employment and periodic clinical examinations of this organized sector of the community is also emphasized.
Abstract
This study was conducted with the aim of identifying the frequency and risk factors of severe schistosomal morbidity in rural Alexandria, and evaluating the role of procollagen III peptide, a connective tissue matrix marker, in predicting schistosomal morbidity. Abis 4 village was randomly selected as the study area. All 2577 households were asked to provide morning stool samples, and the intensity of infection was calculated. An estimated sample size of 1082 subjects was randomly selected, interviewed and examined. Adults with clinically detected organomegaly and an equal number of healthy villagers were referred for detailed investigations. A 5-item portal hypertension score was developed consisting of portal vein diameter, spleen length, collaterals, direction of portal blood flow and degree of periporal fibrosis. Subjects recording 2 or more abnormal findings of these items were considered cases that developed severe morbidity, while the remaining group was taken as a control. A randomly selected sample of children was also referred to the hospital for detailed investigations. Children with an enlarged left lobe of the liver and with periportal fibrosis more than 3 mm were considered cases that developed early morbidity, and the remaining group of children were considered controls.

Results
The overall prevalence Schistosoma mansoni accounted for 20.5%, but with a low intensity of infection. Seropositivity of HCV antibodies was found to be strikingly high (45.9%) in adults >35 years old with clinically detected organomegaly. Older age and heavy water canal exposure were the only significant risk factors for developing clinically detectable organomegaly. Previous viral infection (anti-HCV and/or anti-HbsAg) was the only significant risk factor for developing high procollagen levels, portal hypertension score 2, and AST/ALT ratios greater than 1 (i.e. severe morbidity). High procollagen levels were also predicting severe portal hypertension and high AST/ALT ratios. Regarding children, older age and positive stool analysis were the only determinants of developing high procollagen levels, while schistosomal infection proved to be the only significant risk factor for developing early liver morbidity.

Conclusion
This study revealed that S. mansoni and viral hepatitis infections are the main causes of chronic liver diseases in Egypt. The risk of concomitant viral infection should therefore be emphasized in planning preventive programmes.

Publications

Background
Schistosomal morbidity appears to vary from one geographical area to another, being related to the strain of parasite, the host and the environment. It is widely accepted that most persons with S. mansoni infection in the same schistosomal endemic area, living in similar environmental conditions, and probably with comparable frequency of
water canal exposure, show different degrees of morbidity. Several factors may contribute to its pathogenesis including the intensity and duration of infection, genetic predisposition, blood group, and timing of treatment. Schistosomal morbidity can be assessed by physical examination, laboratory investigations and ultrasonography. Recently, connective tissue matrix markers were used as predictors of the degree of fibrosis. Periportal hepatic fibrosis (PPF) is a main sequel of schistosomal infection, however, the degree of fibrosis cannot be exactly foretold. Some patients may progress to the late stages of shrunken liver, while others may stop at an intermediate stage of hepatic fibrosis.

This study was conducted aiming at the identification of the frequency and risk factors of severe schistosomal morbidity in rural Alexandria, in addition to the evaluation of the role of procollagen III peptide, a connective tissue matrix marker, in predicting schistosomal morbidity.

**Materials and methods**

A map of the Abis villages in rural Alexandria was prepared, and Abis 4 village was randomly selected as the study area. All 2577 households were asked to provide morning stool samples and the number of eggs per gram of faeces (epg) was determined by averaging egg counts on 3 modified Kato thick smears. An estimated sample size of 1082 subjects was selected by a systematic random sampling method. Subjects were interviewed using a pre-tested questionnaire. The information collected included: sociodemographic, lifestyle, history of antischistosomal treatment and medical past history. Clinical examination was carried out in the field. A scoring system for water canal exposure was developed taking into consideration the nature, frequency and duration of exposure.

**Morbidity study in adults** Adults with clinically detected organomegaly and an equal number of healthy villagers were referred for liver function tests, procollagen type III peptide, HCV-antibodies, HbsAg, and abdominal Doppler ultrasonography. A 5-item portal hypertension score was developed consisting of: portal diameter >13mm, spleen length >120mm, presence of collaterals, hepatofugal direction of portal blood flow and periportal fibrosis ≥ grade 2. Subjects recording 2 or more of these items (portal hypertension score ≥2) were considered cases that developed severe morbidity, while the remaining group was taken as a control.

**Morbidity study in children** Randomly selected children were referred to perform the same investigations previously described for adults. Children with an enlarged left lobe liver and periportal fibrosis more than grade 1 (≥3mm) were considered cases that developed early morbidity, and the remaining group of children were considered controls.

**Main study findings**

*S. mansoni* is still endemic in this area, with an overall prevalence of 20.5%. Its prevalence increased with age to reach to a plateau at 15-30 years of age, then was nearly stable thereafter even in older individuals. The intensity of infection was low according to the WHO staging system (geometric mean less than 100 epg). Males recorded higher prevalence and intensity of infection compared to females.

Factors potentially affecting schistosomal morbidity were studied and these included: age, sex, intensity of infection, water canal exposure habits, education, antischistosomal treatment, past history of comorbid liver conditions and risk factors for liver morbidity.

This study revealed that *S. mansoni* and viral hepatitis infections are the main causes of chronic liver diseases in Egypt. The seropositivity of HCV antibodies was found to be strikingly high in adults >35 years old; in fact, age was the only significant determinant of HCV seropositivity in this study. Clinical examination proved to be a nonreliable method with low sensitivity, low negative predictive value, moderate specificity and high positive predictive value for detection of morbidity. In spite of these limitations, older age and heavy water canal exposure were the only significant risk factors for developing clinically detectable organomegaly. Previous viral infection (anti-HCV and/or anti-HbsAg) was the only significant risk factor for developing high procollagen levels, portal hypertension score ≥2, and AST/ALT ratio of more than 1 (i.e. severe morbidity). High procollagen levels were also predicting severe portal hypertension and high AST/ALT ratios.

Regarding children, age (older than 10 years) and positive stool analysis were the only significant risk factors for developing high procollagen levels. Positive stool analysis proved to be the only significant risk factor for developing early morbidity (enlarged left lobe liver and PPF >3mm).

The preventive strategies against the historical public health challenge of schistosomiasis in Egyptian villagers is expected to change dramatically with increasing awareness about the risk of concomitant viral infections.
**Abstract**

This project aimed at studying the effect of the involvement of members of the Iraqi Women's Federation (IWF) on the outcome of the strategy of directly observed treatment short course (DOTS) in the treatment of tuberculosis patients. A total of 172 newly diagnosed cases in Saddam City, Baghdad, were systematically randomized into 2 groups, one using the conventional method of administering DOTS in primary health care centres, and the other with involvement of members of the IWF supervising DOTS.

**Results**

Involvement of the IWF had a significant impact on the outcome of the DOTS strategy for tuberculosis treatment. The cure rate in the intervention group was 83.7%, compared to 68.6% in the control group. All patients in the intervention group were compliant, compared to 86% in the control group. The cure rate for patients was found to be significantly negatively associated with the number of doses missed, and the cut-off point was found to be 8 doses. Smear conversion rates were significantly higher in the intervention group compared to the control group. On the other hand, neither the defaulter rate nor mortality was affected by this intervention.

**Conclusion**

This study provides evidence that involvement of nongovernmental organizations would significantly improve the treatment outcome of tuberculosis patients under the DOTS strategy. The introduction of this intervention in the National Tuberculosis Control Programme is highly recommended.

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**Background**

There have been several reports regarding the involvement of nongovernmental organizations (NGOs) in home visiting of tuberculosis patients in order to supervise the directly observed chemotherapy short course, DOTS. Moreover, NGOs have collaborated with national tuberculosis programmes in DOTS implementation, health education, contact tracing, defaulter retrieving, advocacy and financial support to tuberculosis patients. This study aimed at evaluating the impact of the involvement of the Iraqi Women's Federation (IWF) in improving the DOTS outcome compared to the conventional method of DOTS administration.

**Material and methods**

An intervention study was conducted from February to December 2001 in the Primary Health Care Centres of Saddam City, Baghdad. A total of 172 newly diagnosed cases were systematically randomized into intervention or control groups (86 in each group). Diagnosis took place in the Tuberculosis Institute, Baghdad, based on 3 consecutive positive sputum smears by Ziehl-Neelsen Stain.

**Intervention**

Twenty members of the IWF were nominated and selected from the local branch of the IWF in Saddam City and trained for 2 days on the problem of tuberculosis and on the direct daily close supervision of DOTS. Training included how to approach patients daily and how to administer the antituberculosis drugs directly from their hands during the 2 months of intense treatment of DOTS. They were trained on the adherence to the visit, the schedule of treatment and the provision of direct daily hand-to-mouth

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**Conclusions and implications of the study**

- This study reported the beneficial impact of involving nongovernmental organizations on the outcome of tuberculosis patients managed under the DOTS strategy.
- There is a need to implement this successful intervention on a wider scale in the country and in other countries where tuberculosis is a public health problem.
- A minimum of 8 missed doses of anti-tuberculous drugs could significantly affect the cure of patients.
therapy for patients assigned to them; thereafter, they were responsible for their patients' monthly follow-up. They were also taught to educate the patient and his family on the disease and its transmission, and trained in the completion of the questionnaires. These individuals were strictly observed by the tuberculosis coordinator. After the period of intensive treatment using the same regimen (rifampicin, isoniazid, pyrazinamide, ethambutol or streptomycin), the patients were referred to the tuberculosis coordinator to complete their treatment of daily rifampicin and isoniazid administered by the nearest primary health care centre.

The conventional method of delivering antituberculosis drugs to the patients took place in the primary health care centre for a 6-month period, during which the drugs were delivered on a daily basis to the 86 patients belonging to the control group. Patients from the two groups were followed-up in a timely fashion to measure the smear conversion rates at the end of the first, second, third and fifth months, the defaulter rates, non-compliance, cure rates, treatment failures and mortality rates.

Main study findings

There were no significant differences between the intervention and control groups regarding age, gender, type of family and occupation. On the other hand, house ownership was significantly higher in the intervention group, and the crowding index was significantly higher in the control group. In more than one-third of patients, a household index case was identified, mainly a second-degree relative, compared to almost one-quarter of patients with a non-household index case. The ratio of household to non-household index cases was 1.64.

The cure rate was significantly higher in the intervention group (83.7%), compared to the control group (68.6%).

The risk of being smear-positive was significantly higher through the period of follow-up in the control group compared to the intervention group. Alternatively, the seroconversion rates were significantly higher in the intervention group compared to the control group, and showed a significant trend with the duration of follow-up.

Moreover, the noncompliance problem was absent from the intervention group supervised by the members of the IWF. In the control group, noncompliance to treatment varied in duration according to the duration of follow-up, but the overall noncompliance rate was 86%. Noncompliance in this group was not associated with gender, family type, ownership of a house or marital status. On the other hand, the duration of follow-up was the only significant determinant of noncompliance, and noncompliance was significantly associated with treatment failure.

In fact, the cure of a patient was found to be negatively associated with the number of doses missed (P = 0.03), and the cut-off point was found to be 8 doses (P = 0.04).

The defaulter and the mortality rates were not significantly different among the 2 groups in this study. Defaulter rates in the two groups were 11.18% and 10.6% in the intervention and control groups, respectively. Regarding mortality, 2 cases died, 1 in each group (1.2%). However, a larger sample size is needed before drawing conclusions about defaulting and mortality rates. This is one of the study limitations.

Conclusions and recommendations

This study provides evidence that involvement of nongovernmental organizations would significantly improve the cure rate, smear conversion rate and the compliance rate of pulmonary tuberculosis patients, thus reducing infectivity days, preventing the spread of the disease at the community level, and reducing relapses.

Research on the cost-effectiveness of this intervention is recommended in order to provide additional evidence about the importance of its introduction within the DOTS strategy in low-income countries with a heavy tuberculosis burden.
Does routine home visiting improve the return of late-coming patients?

Iraq
Baghdad

Study period: May 2001–2002

Small Grants Scheme (SGS) 2000 No. 35

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Conclusions and implications of the study

- Home visiting of patients by trained personnel has a significant positive impact on the return of latecomers to continue their medication, and on their compliance and treatment outcomes.
- Risk factors for noncompliance were: positive family history of tuberculosis, residing at a far distance from tuberculosis centres, social stigma, economic burden, feeling no improvement with treatment, and negative attitude towards drug intake.
- Adherence to treatment was not found to be a significant predictor of treatment failure and mortality from tuberculosis. Other determinants should be carefully investigated.
- Home visiting of patients by trained personnel should be an integral part of DOTS strategy.

Abstract
This study aimed at evaluating the impact of home visits on the compliance and treatment outcomes of tuberculosis patients who collected their drugs late. Four hundred and eighty new smear-positive patients who collected their drugs late from health centres over a period of 6 months were selected and randomized for either home visiting or non-home visiting.

Results
Home visiting was highly effective in improving the return of late-comers; out of 240 patients, 231 returned and 9 did not return. The treatment success rate was 94.1% compared to 76.7% in the control group. The defaulter rate in the intervention group was 0.9% while in the control group it was 10%. Smear conversion at the end of the treatment was better in the intervention group (92.9%) compared to the control group (75%).

Conclusion
Home visits by trained personnel would significantly improve the compliance of patients, treatment success rate and smear conversion rate and would reduce the defaulter rate.

Background
Successful completion of treatment for active tuberculosis patients is the single most important way to control and prevent new cases. However, treatment completion is often delayed or unsuccessful because it requires patients to adhere to taking medication for at least 6 months. A successful, cost-effective, community-based programme of directly observed therapy, utilizing volunteers, clinic and community health workers, has helped ensure adherence to therapy.

In Iraq tuberculosis is a major public health problem. In spite of the 50% increase in notification rate achieved during the last 4 years, the defaulter and latecomer rates are relatively high (10% and 20%, respectively).

This study was designed to evaluate the effect of home visits on the return of latecomers and the treatment success rate in comparison to the conventional method of directly observed therapy, short course (DOTS).

Materials and methods
This was an intervention study conducted in 15 randomly selected health centres in Baghdad. A total of 480 patients who collected their drugs late were randomized into either home visiting (intervention group = 240) or non-home visiting (control group = 240). Smear-positive patients were selected due to their role in the spread of infection and the fact that treatment outcomes are more accurately evaluated by following smear conversion.

Fifteen home visitors were trained on motivating late comers to adhere to the prescribed regimen. A group of TB coordinators, each for one district, were also trained on the identification of late comers, on how to send home visitors and on the follow-up of the project’s implementation according to the study design. They were also trained on data recording in order to minimize errors in registration in treatment cards and books. The records of home visiting were filled in separate sheets away from the patients’ records.

Another person was identified and
trained to fill the data collection form. This person was blinded about home visiting in order to reduce the possibility of bias. The study was carried out after receiving ethical clearance and all individual information was strictly confidential.

The two groups were matched for the distance from the TB center in order to avoid its confounding effect. Several constraints were met during conducting the study such as refusal of home visiting by the patients, their absence during the time of the visit, or incorrect addresses. These constraints were faced by educating the patients about the importance of these visits, in case of refusal, or asking the home visitor to pay another visit to the patient, in case of his absence during the initial visit. On the other hand, it was not possible to retrieve the patients with incorrect addresses and this group was excluded from the study.

**Definitions**:

**Latecomer**: Any patient who does not come to take medication for at least 3 days after a scheduled appointment. The patient is defined as a latecomer on the first episode of coming late.

**Home visiting** Visit by a person trained and assigned to visit the home of a latecomer in order to motivate him/her to attend the health centre daily.

**Data collection** The home visitors were trained to visit latecomers under the guidance of the tuberculosis coordinator. They received standardized instructions. The treatment completion was recorded from the tuberculosis register and from patients' treatment cards at the tuberculosis centre.

The information was checked before entry to the computer, edited and double entry was performed in order to reduce the probability of error.

**Data analysis** Chi-square test was used to compare between the intervention and control groups regarding compliance to treatment, return of latecomers and treatment outcome.

### Main study findings

Cases were found to be comparable to the controls and there was no significant difference between the two groups in terms of age, gender and marital status. It is clear from the findings of this study that home visiting of patients had a significant impact on the return of latecomers to continue their medication. Out of 240 patients, 231 (96.2%) returned and 9 (3.8%) did not. Regarding the treatment success rate, it was significantly higher in the intervention group compared to the control group (94.1% and 76.7%, respectively).

Home visiting also had a significant impact in reducing defaulter rates (0.9% and 10%, in the intervention and control groups, respectively).

Although the failure rates and death rates were reduced by this intervention, there was no significant difference between the two studied groups. The failure rate was 5.8% in the control group compared to 2% in the intervention group. The death rate was also higher in the control group (3.4% versus 1.3%, p>0.05).

Noncompliance was found to be significantly associated with positive family history, living far from tuberculosis centres, social stigma, economic burden, feeling of non-improvement with treatment, and refusal to take drugs.

Therefore, home visiting proved to play an important role in improving compliance and the treatment outcome of new smear-positive pulmonary tuberculosis patients.

### Conclusions and recommendations

Home visiting by trained personnel would significantly improve the compliance of patients, the success rate, smear conversion rate and defaulter rate. Accordingly, home visiting could play a major role in reducing the infection rate in the community.

The integration of this successful intervention in DOTS strategy is highly recommended.
Abstract
This study aimed at evaluating the impact of home visits on the compliance and treatment outcomes of tuberculosis patients who collected their drugs late. Four hundred and eighty new smear positive cases who collected their drugs late from health centres over a period of 6 months were selected and randomized for either home visiting or non-home visiting.

Results
Home visiting was highly effective in improving the return of late-coming patients, recording a compliance rate of 89% compared to 80% among the group that did not receive home visiting. Other determinants of treatment compliance were studied but none had a significant impact on compliance. Non-adherence to treatment was attributed to the severity of illness, stigma, worries about teratogenic effects on fetuses, losing treatment cards, family circumstances and fear of deportation in the case of refugees.

Conclusion
Home visiting by trained personnel would significantly improve the compliance of patients, and consequently treatment success rates, smear conversion rates and would reduce defaulter rates.

Background
With an annual risk of infection of 3%, tuberculosis represents a major public health problem in Djibouti. This problem is aggravated by the influx of immigrants from neighbouring countries, who represent 50% of smear-positive pulmonary tuberculosis cases and 40% of all cases. Since 1990, several conferences and workshops were held in order to coordinate tuberculosis control activities within the Horn of Africa. Adherence to treatment is the cornerstone for achieving higher cure rates, hence tuberculosis control. However, the defaulter rate had reached 20%, representing a serious impediment to tuberculosis control in the country, besides favouring the spread of multidrug resistant strains.

This study was designed to evaluate the effect of home visiting on the return of latecomers, and thus, on the treatment success rate of tuberculosis patients in Djibouti.

Materials and methods
An intervention study was conducted in 6 tuberculosis centres in Djibouti. A total of 480 patients who collected their drugs late were randomized into either home visiting (intervention group = 240) or non-home visiting (control group = 240). Smear-positive cases were selected due to their role in the spread of infection and the fact that treatment outcome is more accurately evaluated by following smear conversion.

Home visitors were trained on motivating late comers to adhere to the prescribed regimen. A group of Tuberculosis coordinators were also trained on the identification of late comers, on how to send home visitors and on the follow-up of the project's implementation according to the study design. They were also trained on data recording in order to minimize errors in registration in treatment cards and books. The records of home visiting were filled in separate sheets away from the patients' records. Another person was identified and trained to fill the data collection form. This person was blinded about home visiting.

Conclusions and implications of the study
- Home visiting of patients by trained personnel has a significant positive impact on the return of latecomers to continue their medication, and on their compliance and treatment outcome.
- Noncompliance was justified by: severity of illness, social stigma; economic burden; worries about teratogenic effects on fetuses, losing treatment cards, family circumstances (death, illness, etc.), fear of deportation in case of refugees.
- Home visiting of patients by trained personnel should be an integral part of DOTS strategy.
in order to reduce the possibility of bias. The study was carried out after receiving ethical clearance and all individual information was strictly confidential. The two groups were matched for the distance from the TB center in order to avoid its confounding effect.

**Definitions:**

**Latecomer:** Any patient who does not come to take medication for at least 3 days after scheduled appointment. The patient is defined as a latecomer on the first episode of coming late.

**Home visiting:** Visit by a person trained and assigned to visit the home of a latecomer in order to motivate him/her to attend the health centre daily.

**Data collection:** The home visitors were trained to visit latecomers under the guidance of the tuberculosis coordinator. They received standardized instructions. Treatment completion was recorded from the tuberculosis register and the patients’ treatment cards at the tuberculosis centre.

**Data analysis** Chi-square test was used to compare between the intervention and control groups regarding compliance to treatment and return of latecomers.

**Main study findings**

Seventy-five percent of the study population was younger than 35 years of age. There was no significant difference between the intervention and the control group regarding: age, sex, residence, nationality, income, existence of household contacts, and the presence of co-morbid conditions. The intervention group had significantly lower educational and occupational levels and were living at closer distances from the tuberculosis centres.

The results of this study showed that home visiting of patients had a significant impact on the return of latecomers to continue their medication. Adherence to treatment was significantly higher in the intervention group compared to the control group (89% and 80%, respectively).

Other determinants of treatment compliance were studied but none had a significant impact on compliance. These factors were: sociodemographics, educational level, occupation, income, existence of contacts, and distance to the tuberculosis centre. However, these negative findings could be explained by the fact that 90% of the study population was living within less than 2 km from the tuberculosis centres. In addition, they belonged to a relatively homogenous social class and were exposed to similar environmental and social conditions. While apparently reassuring, these negative findings indicate that only a small proportion of the population is served by the tuberculosis centres, leaving the majority of the population, nomads or those living in remote areas, unserved.

Patients justified their non-adherence to treatment by: severity of illness, stigma, worries about teratogenic effects on fetuses, losing treatment cards, family circumstances (death, illness, etc.), and fear of deportation in the case of refugees.

**Conclusions and recommendations**

Home visiting by trained personnel would significantly improve the compliance of patients, success rate, smear conversion rate and defaulter rate. Accordingly, home visiting could play a major role in reducing the infection rate in the community. The integration of this successful intervention in DOTS strategy is highly recommended.
Abstract
A case-control study conducted in all tuberculosis centres in Egyptian governorates aimed at investigating the risk factors of treatment failure in Egypt. One hundred and nineteen new smear-positive patients recording treatment failure at the end of their treatment period were enrolled in the study, these were the study cases. An equal-sized group of cured patients, matched with these new patients in age, gender, place and time of treatment constituted the control group.

Both the cases and controls were compared regarding socio-demographic variables, compliance to treatment, knowledge regarding tuberculosis, accessibility to health care, services provided in the health facility, family knowledge and support given to the patient.

Results
Significant risk factors for treatment failure were: missed days (1.4-fold increased risk for each missed day of treatment), diabetes mellitus, poor patient knowledge regarding the disease and deficient health education. Missing at least 8 days of treatment would significantly affect the treatment outcome and was associated with a 3-fold increase in the risk of treatment failure.

The most alarming finding of this study is that 40% of cases with treatment failure were fully adherent to the treatment regimen. Risk factors for treatment failure in this compliant group were diabetes mellitus (10-fold increased risk) and poor patient knowledge regarding the disease (9-fold increased risk). On the other hand, there was a significantly lower risk for treatment failure among females and for those with a high number of children in the family. These factors explained only 46% of the variability in treatment failure. Other causes for treatment failure should be therefore investigated in this community such as drug resistance and drug quality.

Conclusion
These results emphasize the need to strengthen the supervision of treatment compliance, proper control of diabetes, proper health education, and timely and high quality care to all patients.

Background
The National Tuberculosis Control Programme (NTP) in Egypt was launched in 1989 in Cairo and Giza, and subsequently extended to the entire country by the year 1999. The Directly Observed Treatment Short course (DOTS) strategy started on a pilot basis in 1996 and reached 100% national coverage in December 2000. Case management according to DOTS entailed regular daily observation of treatment in the first 2 months of the initial phase, followed by weekly observation during the patient’s visit to the treating health centre, where the patient swallows his/her medicine and is given medications sufficient for the next 6 days. Home visits are carried out for non-compliant patients. Therefore, upon completion, patients should record a total of 76 visits to the centre. The treatment regimen consists of 2S(E)HRZ/4HR, as recommended by the national tuberculosis control guidelines.

Treatment failure is a serious problem for tuberculosis control programmes in many countries throughout the world. These patients tend to have higher mortality. Moreover, they remain infectious and hence transmit the disease to other members of the community. In Egypt, treatment failure represents 3 to

Conclusions and implications of the study
- Missing at least 8 days of treatment significantly affects the treatment outcome, indicating the need to strengthen supervision throughout the treatment period of tuberculosis patients.
- Poor patient knowledge regarding tuberculosis proved to be a significant predictor of treatment failure, indicating deficient health education delivered to patients. Strengthening health education in DOTS strategy is therefore recommended.
- Diabetic patients proved to be at a significantly higher risk of treatment failure and should be subjected to tight blood sugar control and supervision. These results suggest shifting from oral antidiabetic drugs to insulin for tuberculosis patients until treatment completion.
5% of new smear-positive cases and 13 to 17% of re-treated cases. This study was carried out to investigate the risk factors of treatment failure in Egypt.

**Materials and methods**

A case-control study was conducted in all tuberculosis centres in Egyptian governorates that recorded treatment failure. All new smear-positive patients recording treatment failure as a treatment outcome in the tuberculosis centres of all governorates were enrolled in the study and labelled as study cases. A control group equal in size to the new cases consisted of patients who were declared cured at the end of their treatment. Controls were matched to cases with regard to age, gender, place of treatment and time of treatment.

Both cases and controls were interviewed regarding socio-demographic variables, compliance to treatment, and knowledge regarding tuberculosis and its medication. The interviews also covered accessibility to health care and service provided in the health facility. A family member was interviewed about family knowledge and support given to the patient. The collected information was cross-checked with the tuberculosis register, the treatment card and the supervising nurse.

**Main study findings**

One hundred and nineteen patients with treatment failure were enrolled during the study period, which represents a prevalence of 2.9% (119 treatment failures out of 4181 total tuberculosis cases). There was no significant difference between governorates regarding the prevalence of treatment failure, which ranged from 1% to 5%.

As controls were matched to cases, there was no significant difference between them regarding socio-demographic variables and treatment time. Two-thirds of cases and controls were males, and more than half of each group were 25 to 45 years old. Sixty percent of each group were illiterate and around half of each group were unemployed.

Regarding the accessibility to health services, 27% of patients with treatment failure were living at a distance of 10 km or more from the tuberculosis centre compared to 11% of cured patients, and this was statistically significant. Diabetes mellitus was the only co-morbid condition recorded at a significantly higher percentage among cases compared to the controls (26% and 7.6%, respectively).

Evaluating patients’ knowledge regarding tuberculosis revealed a significantly lower level of knowledge among failed cases; none were rated as having very good knowledge compared to 21% of cured cases. Expectedly, there was a significant difference between both groups regarding the frequency of health education sessions. The source of information was mainly the chest hospital and TV.

Adverse treatment effects were recorded in 30% and 23% of failed cases and controls, respectively (p>0.05). Failed cases were less satisfied with the quality of care delivered in the health facilities and by the providers. Almost one quarter of this group reported waiting for more than half an hour to receive care.

In addition, families of failed cases were smaller in size and recorded significantly lower knowledge scores regarding the disease and its treatment compared to those of cured patients.

Regarding irregular treatment patterns, there was a significant difference in the number of missed doses between cases and the controls during the second, third, fourth and sixth months of treatment.

Multivariate logistic regression analysis showed that the significant risk factors for treatment failure were missed days (1.4-fold increased risk for each missed day of treatment), the presence of diabetes mellitus, poor patient knowledge regarding the disease, and deficient health education. Missing at least 8 days of treatment significantly affected the treatment outcome and was associated with a 3-fold increase in the risk of treatment failure.

The most alarming finding of this study is that 40% of patients with treatment failure were adherent to the treatment regimen. Risk factors for treatment failure in this compliant group were diabetes mellitus (10-fold increased risk) and poor patient knowledge regarding the disease (9-fold increased risk). On the other hand, there was a significantly lower risk for treatment failure among females and among those with a high number of children in the family. However, these factors explained only 46% of the variability in treatment failure. Other causes for treatment failure that were not investigated in the present study should therefore be investigated in this community, such as drug resistance and drug quality.

Among the non-compliant group, apart from the invariable implication of poor knowledge of the causes of their condition, other risk factors emerged such as the distance to the tuberculosis centre (1.4-fold increased risk for each kilometre from the tuberculosis centre) and poor satisfaction with care. In addition, younger age and small family size proved to be significant predictors of treatment failure.
Abstract
A cross-sectional study was carried out in 2001 among registered medical practitioners in the public and private health sectors in the north-west zone of Somalia. The aim of the study was to assess the knowledge and practices of medical practitioners in the diagnosis and management of tuberculosis using a structured questionnaire.

Results
Only 53 out of 100 registered doctors could be interviewed. Seventeen reported working solely in the private sector, 7 in the public sector and 29 in both. Symptoms were correctly identified by 66% of the doctors, and 60% indicated sputum smear microscopy as the most important diagnostic test. Only one practitioner had notified a patient to the National Tuberculosis Programme, and very few practitioners prescribed the correct regimen or advocated direct observation of drug taking. Private practitioners were twice as likely to have suboptimal knowledge regarding pulmonary tuberculosis diagnosis as those working in the public and private sectors.

Conclusion
Tuberculosis patients are mainly managed in the private sector, and few doctors follow the guidelines of the National Tuberculosis Programme. The distribution of the NTP guidelines to all medical practitioners in the North-west Zone, together with the regular training of doctors in the diagnosis and case management of tuberculosis were the main study recommendations.

Impact of research results on health policy
A workshop on tuberculosis control for private practitioners was held in Hargeisa on 3-4 November 2002. The meeting was very successful with active participation of all attending doctors. At the end of the meeting, the Minister of Health and Labour issued a Ministerial Decree banning the sale of anti-tuberculosis drugs in private pharmacies. The KAP study, the workshop and the interest of the Minister of Health should have a beneficial impact and improve the situation of tuberculosis control in the country.

Background
In the North-west zone of Somalia, the private sector contributes significantly in the provision of health care. While many tuberculosis patients are treated within the National Tuberculosis Programme (NTP), observations at tuberculosis clinics have indicated that tuberculosis patients coming from the private sector were not given treatment in line with the national tuberculosis guidelines. However, little is known about the knowledge and practices of the medical doctors in the North-west zone. Therefore, this study was undertaken to evaluate the knowledge and practices of medical practitioners regarding the symptoms, diagnosis and case management of pulmonary tuberculosis in the North-west zone.

Materials and methods
The study was a cross-sectional survey interviewing medical practitioners by a structured questionnaire, and took place between July and November 2001. The eligible population included all the qualified medical practitioners in Somaliland. A list of all medical doctors registered by the Government in the north-west zone of Somalia was obtained from the Ministry.
of Health and Labor (2001), including public and private practitioners. These consisted of 100 medical practitioners after excluding medical practitioners working for the NTP (7) or had no clinical work. Among this group, only 53 participated in the study. Causes for non-participation was the busy practice (4), travelling abroad (6), mental handicap (3), or inaccessibility due to presence of the health facility in the war-zone (10).

The questionnaire was prepared in English, and inquiring about general information of the responding doctors and their qualifications, number of patients seen and treated in the last 12 months. Questions about the knowledge of the symptoms of pulmonary tuberculosis (PTB) were regarded correct if they identified 3 of 6 major symptoms of PTB mentioned in the NTP Guidelines. The knowledge of diagnostic procedures was regarded correct if they ranked sputum microscopy higher than X-ray and other laboratory examinations. The regimen for smear positive PTB cases was regarded correct if following the NTP guidelines (2HREZ/4HR). They were also inquired about supervision of the drug taking, tracing of patients who missed appointments, tracing of family contacts, and notification of their TB patients. The questionnaire was pre-tested and modified according to the results of the pilot phase. Data collection was done by two doctors. All towns were visited except Lasanod, which could not be reached for security reasons.

Data management and analysis The two interviewers scrutinized each other’s filled questionnaire before data entry. Data entry was done in Epi-Info version 6.04 by a team of 2 doctors and a data-clerk. Data entry validation was done. Cleaning of data was done before analysis using descriptive statistics. The potential risk factors for sub-optimal knowledge of symptoms and diagnosis were identified by computing their relative risks and corresponding 95% confidence intervals.

Ethical considerations Ethical clearance was obtained from the Ministry of Health. Written informed consent was obtained from each doctor before the interview, and the collected information was confidential.

Main study findings

Of 100 registered medical practitioners, only 53 doctors could be interviewed. Seventeen medical practitioners reported working solely in the private sector, 7 in the public sector and 29 in both. Sixty percent of the respondents had treated a tuberculosis patient within the last 12 months, 66% indicated a correct combination of symptoms, and 60% indicated the correct diagnostic tests, but only 1 doctor had submitted notification to the NTP. Only 7% of the doctors were able to define the correct regimen for tuberculosis patients; 45% prescribed anti-tuberculosis drugs on a daily basis, 13% weekly and 32% monthly. DOTS was performed by only 13% of the doctors.

The majority of doctors (71.7%) indicated that PTB was suspected if the duration of a cough was 4 weeks or more, 11 doctors (20.8%) indicated that patients who missed their appointments should be traced, and 6 would try to look for tuberculosis among the contacts of the patients. Only 2 reported that they had submitted a report to the NTP after treating tuberculosis patients.

Practising in the private sector was a significant predictor for suboptimal knowledge of diagnosis of PTB compared to practicing in both private and public sectors (RR 2.1, 95% CI 1.1-4.3).

Conclusions and recommendations

A considerable proportion of tuberculosis patients are managed in the private sector and few doctors follow the guidelines of the National Tuberculosis Programme of Somalia. Training of medical practitioners in the diagnosis and case management of tuberculosis patients is needed to improve tuberculosis control in the north-west zone of Somalia. Distribution of the NTP guidelines to all medical practitioners in this zone was also recommended.
Abstract
A cross-sectional study was carried out in 2001 among a sample of 245 registered private medical practitioners in Rawalpindi and Lahore, Pakistan. The aim of the study was to assess the knowledge and practices of private medical practitioners in the diagnosis and management of pulmonary tuberculosis using a structured questionnaire.

Results
Fewer than 1% of private medical practitioners are aware that coughing for more than 3 weeks is the main diagnostic symptom suggesting pulmonary tuberculosis (PTB), and that sputum microscopy is the diagnostic test for a PTB patient. And in spite of the fact that very few doctors have sputum examination facilities at their clinics and are unable to deliver drugs in their private practice, only a small proportion refer patients to public tuberculosis centres.

None of the private medical practitioners follow national tuberculosis control guidelines in prescribing drugs, and a majority admit an inability to categorize a PTB patient for treatment. Furthermore, none ensure the intake of anti-tuberculosis medicines under the supervision of a doctor or a health worker. Most of them ensure this by personal counselling, clinical assessment and through relatives, and the outcome of treatment is mainly determined by clinical assessment rather than by sputum microscopy.

Very few private medical practitioners are maintaining records of pulmonary tuberculosis patients or are trying to contact non-compliers or defaulters. Contacts tracing is performed by enquiring about symptoms instead of tuberculin testing.

Conclusion
Private medical practitioners are not following national tuberculosis control guidelines in Pakistan in diagnosing, treating and conducting follow-up of pulmonary tuberculosis patients. The study revealed the need to subject the private sector to continuous training on the national tuberculosis control guidelines, as well as the need for monitoring during the whole process of diagnosis, treatment and follow-up by the National Tuberculosis Control Programme.

Background
In spite of the fact that the private health sector has grown considerably in the last few decades, information on the extent and role of the private sector in tuberculosis care tends to be very sparse. Limited literature is available, especially from Pakistan, on the subject of knowledge and practices of private medical practitioners in the diagnosis, treatment and follow-up of pulmonary tuberculosis (PTB) patients. The aim of this study was to collect relevant and accurate information regarding the knowledge and practices of the general medical practitioners of the diagnosis, treatment and follow-up of PTB patients, and to employ this baseline information to plan future interventions in order to improve tuberculosis care provided by private medical practitioners.

Conclusions and implications of the study
Private medical practitioners are not following national tuberculosis control guidelines in Pakistan in diagnosing, treating and conducting follow-up of pulmonary tuberculosis patients. This leads to inadequate management of tuberculosis patients who remain a dangerous source of infection in the community.

Only 1 out of 245 medical practitioners mentioned coughing for more than 3 weeks as the main symptom suggestive of pulmonary tuberculosis. Similarly, only 1 practitioner recommended sputum microscopy as the basis for diagnosing tuberculosis. These are alarming findings indicating inappropriate case detection in the private sector. These two particular issues could be the main theme of an awareness campaign for early case detection.

The private medical practitioners should be trained on the national tuberculosis control guidelines. Moreover, functional collaboration needs to be established between private medical practitioners and the National Tuberculosis Control Programme to provide quality tuberculosis care services. Finally, the overall process of diagnosis, treatment and follow-up of pulmonary tuberculosis patients in the private sector should be closely monitored.
Materials and methods
A descriptive cross-sectional survey was conducted in 2 major cities in Pakistan, Rawalpindi and Lahore, to determine the knowledge and practices of private medical practitioners. The study subjects were the qualified medical graduates who were practising medicine on a full- or part-time basis outside the government (public) sector. A comprehensive list of all private medical practitioners was obtained from the medical representative of Wythe-Leaderle. Five hundred and eighty two were in Lahore and 302 in Rawalpindi. The basic criterion for inclusion in the study was that the private medical practitioners must have provided cure to at least 1 PTB patient during the last year.

Out of the 884 private medical practitioners in the two cities, a sample of 245 was interviewed using a semi-structured study tool. The sample included 37 postgraduates and 48 women. Most of them (86%) had provided treatment to 1-10 PTB patients during last 3 months.

Main study findings
Less than 1% (1 out of 245) of private medical practitioners are aware that coughing for more than 3 weeks alone is the main symptom suggesting pulmonary tuberculosis. A combination of symptoms was mentioned by the rest of practitioners as the main symptoms.

The majority (81%) self-diagnose the patients and only a small proportion refer them to the Tuberculosis Centre.

Less than 1% of private medical practitioners perform sputum microscopy alone in order to diagnose a suspected case of pulmonary tuberculosis.

None of the private medical practitioners take the history of any previous anti-tuberculosis treatment. Most of them give priority to family history and socio-economic status.

The majority (96%) of private medical practitioners cannot categorize a PTB patient for treatment.

None of the private medical practitioners follow the national tuberculosis control guidelines in prescribing drugs. The majority (69%) give a fixed-dose combination (FDC) of 4 drugs, and 27% give 3 to 4 separate drugs initially for 2 to 6 months. During the continuation phase, 42% give a FDC of 4 drugs, and 29% give a FDC of 3 drugs. The majority (89%) give these combinations until the patient is cured. Ninety seven percent of private medical practitioners only write prescriptions to PTB patients because drugs are unavailable in their facilities, 62% prescribe on a fortnightly basis, and 31% on a monthly basis.

None of the private medical practitioners ensure the intake of anti-tuberculosis medicines under the supervision of a doctor or a health worker. Most of them ensure this by personal counseling, clinical assessment and through relatives. Only 3% maintain records of PTB patients.

None of the private medical practitioners assess the effectiveness of anti-tuberculosis treatment through sputum microscopy alone. The majority (76%) evaluate the outcome of treatment only clinically.

Only 2% of private medical practitioners try to contact a patient on anti-tuberculosis treatment if s/he does not return.

Less than 3% of private medical practitioners have sputum examination facilities at their clinics.

Almost all private medical practitioners find it sufficient to enquire only about the symptoms of the close contacts of PTB patients.

The majority of private medical practitioners admit that they do not know about the national tuberculosis control guidelines, and are willing to attend training on the national tuberculosis control guidelines, if provided.

Conclusions and recommendations
Private medical practitioners are not following the national tuberculosis control guidelines in diagnosing, treating and conducting follow-up of pulmonary tuberculosis patients in Pakistan. Training of private medical practitioners in the diagnosis and case management of Tuberculosis patients is recommended.
Abstract
A cross-sectional survey was conducted on private sector physicians in Teheran, Mashhad and Isfahan. A total of 1079 randomly selected physicians completed a standardized questionnaire. The study aimed at determining the knowledge and practices of private sector physicians, both general practitioners and specialists, regarding the diagnosis and management of pulmonary tuberculosis and the adherence of private sector physicians to the National Tuberculosis Programme (NTP) guidelines. This study would enable decision-makers to choose appropriate interventions for more efficient involvement of private sector physicians in tuberculosis control.

Results
Most of the physicians (87.3%) stated sputum smear microscopy as the diagnostic method. Among all private physicians, the correct regimen for new pulmonary tuberculosis (combination, dosage, duration) was stated significantly less frequently than other answers. Fewer than half of the study subjects who treat the patients themselves stated the correct regimen. General practitioners (GPs) had attended more tuberculosis training courses than had specialists. Private physicians who participated in the courses had more correct knowledge of the NTP guidelines and DOTS, and were more aware of the availability of free tuberculosis diagnosis and treatment facilities in the public health system, than those who did not.

Conclusion
The recent introduction of training courses for private physicians in Iran had a beneficial impact on their practices.

Background
One of the main factors hampering efforts in the fight against tuberculosis is the lack of cooperation and coordination between the National Tuberculosis Programme (NTP) and the private health sector. It is believed that most tuberculosis patients initially consult private practitioners. This issue is especially important in large cities. Although tremendous efforts have been made to expand the primary health care network in Iran, health services in large cities are mainly delivered by the private sector. Tuberculosis patients as well as others are mainly managed by private general practitioners (GPs) and specialists in these urban areas of the country. In spite of this fact, the knowledge and practices of private physicians have not been documented through proper studies in Iran. Therefore, this study was conducted to evaluate the knowledge and practices of private sector GPs and specialists in three large cities of Iran in order to provide baseline information for planning future intervention programmes.

Conclusions and implications of the study
- Compared to other countries, a significantly higher proportion of private practitioners in Iran follow NTP guidelines in the diagnosis and management of PTB patients. This was mainly attributed to the recent introduction of tuberculosis training courses in their continuous education. More specifically, good practice was always associated with attendance at tuberculosis control workshops.
- Forty-Two percent of the private sector physicians have attended training courses organized by the Communicable Disease Control Units of the health system at the national or provincial level or in the universities. Course attendance was significantly higher among general practitioners than among specialists. These results emphasize the need for regular training of private physicians in the diagnosis and case management of tuberculosis in order to improve tuberculosis control in Iran.
- Establishing functional collaboration between private medical practitioners and the National Tuberculosis Control Programme was the main study recommendation.

Materials and methods
A cross-sectional survey was conducted among private sector physicians who were randomly selected from a list of private sector physicians (GPs and specialists) prepared by the authorities of the Medical Councils. These physicians
were working in the three largest cities of Iran, each having a population exceeding 1.5 million: Tehran, Mashhad, and Isfahan. Three hundred and eighty, 356, and 343 physicians, recruited from Tehran, Mashhad, and Isfahan, respectively, completed the questionnaires. They were randomly selected from two strata of GPs and specialists through their names and the Medical Council Numbers List, and this method allowed the subjects to be proportionally distributed over the different graduate years of the practising physicians. Considering the fact that drawing conclusions from certain specialities is not relevant to the impact of pulmonary tuberculosis (PTB) training courses, the results focused on GPs and relevant specialities such as paediatrics, infectious diseases, internal medicine and pulmonary medicine.

A total of 732 private physicians were included in the study (599 GPs and 133 specialists).

Data collection was performed through a pre-tested standardized questionnaire that included information about the knowledge and practices of practitioners. This was defined as the agreement of the study subject’s knowledge and practices with the NTP guidelines.

Since the majority of the physicians had attended the tuberculosis training course after the year 1997, the impact of these courses on their practices was also evaluated.

**Main study findings**

Forty-Two percent of the private sector physicians had attended training courses organized by the Communicable Disease Control Units of the health system at the national or provincial level or in the universities. Course attendance was significantly higher among GPs than among specialists.

Out of 712 physicians, 47 (6.6%) did not mention coughing as one of the main manifestations suggestive of pulmonary tuberculosis. Most of the GPs who attended the tuberculosis training courses mentioned a cough duration of 3 weeks to be suggestive of PTB, compared to more than 4 weeks among the specialists. Moreover, a higher proportion of GPs recognized sputum microscopy as the priority diagnostic test compared to specialists. This knowledge was significantly associated with the higher course attendance by GPs over specialists.

Once tuberculosis was confirmed, 82.1% of private physicians referred their patients to public tuberculosis centres.

Half of the physicians stated the correct drug combination and drug dosage. Drug combinations and treatment duration knowledge was significantly higher among GPs than among specialists. The proportion of the physicians who stated the correct drug regimen and attended Tuberculosis Control Workshops was greater than that of those who had attended the course in other institutions. However, among all physicians, the correct regimen was stated significantly less frequently than other answers.

Half of the specialists who treat the patients themselves stated the correct regimen compared to 35% of the GPs.

Regarding the frequency of anti-tuberculosis drug dispensation/prescription for smear-positive patients, most dispense on a monthly basis (41.1%). Daily dispensing was performed in 22% of patients. Physicians who attended the Tuberculosis Control Workshops tended to dispense/prescribe on a daily basis. On the other hand, those who attended the courses in the universities or through the continuing education programmes tended to dispense/prescribe on a monthly basis.

Almost all of the practising physicians ensure the consumption of all dispensed/prescribed drugs. The observer assignment method was more frequently practised among GPs while the paraclinical methods was most frequently used among specialists. The use of the observer assignment method was significantly associated with course attendance, especially of the Tuberculosis Control Workshops.

Documentation of patient information was performed by 78% of private physicians. The majority of private physicians use sputum microscopy to assess the effect of treatment, most have attended the Tuberculosis Control Workshops, and most recognized the correct frequency of sputum examination. Moreover, physicians who attended the courses refer more of their patients to public health system laboratories. Almost all private physicians were aware of the availability of free services.

**Conclusions and recommendations**

Training courses, particularly Tuberculosis control workshops, had a benificial impact on the quality of care delivered by private physicians to tuberculosis patients.

These results emphasize the need for regular training of private physicians on the diagnosis and case management of tuberculosis. More importantly, functional collaboration needs to be established between private medical practitioners and the NTP to provide quality tuberculosis care services, thereby decreasing the burden of tuberculosis in the community.
Operational Research in Tropical Diseases


RESULTS PORTFOLIO
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