REVIEW

RECENT ADVANCES IN VISCERAL LEISHMANIASIS IN CHINA

Guan Li-Ren and Shen Wei-Xia

Institute of Parasitic Diseases, Chinese Academy of Preventive Medicine, Shanghai, People's Republic of China.

Abstract. A review of major advances in investigations and laboratory work on visceral leishmaniasis in China is presented. In the eastern plain and northern mountainous regions, no new cases were detected, while sporadic appearance of the disease was noted in western mountain and desert regions. Considerable achievements were attained regarding epidemiology of visceral leishmaniasis, experimental research on disease control in mountainous regions, sandfly control and elimination as well as immuno-diagnosis of the disease: Noticeable consequences were also obtained from the exploration on the problem of geographical strains and biochemical study of the parasite. The intervention measures in sporadically occurring visceral leishmaniasis in the western mountain and desert regions are discussed.

INTRODUCTION

As a result of implementing extensive intervention begining from the founding of the People's Republic of China, visceral leishmaniasis (VL) which had once been prevalent in 15 provinces/autonomous regions north of the Yangtze River was basically eliminated in plain regions in 1958. In late 1970s, the disease was eradicated in mountain/hill regions of Liaoning, north Hebei, Qinghai and Ningxia provinces/autonomous regions; but still there were reports of new cases from 30 odd counties in Gansu, Xinjiang, Shaanxi, Sichuan, Shanxi and Inner Mongolia provinces/autonomous regions, the affected cases detected annually amounting to 200-300 according to the data issued during the Third National Symposium on Control and Research of Visceral Leishmaniasis, held in November, 1988 in Shanghai. An increase of VL incidence has been noted in south Gansu and north and west parts of Sichuan since 1985, and in 1990 there were 250 newly infected cases found in 11 counties. Control operations aimed at VL are being actively undertaken in affected counties. In parallel with the intervention, investigations and research with the purpose of promoting field work also achieved encouraging results.

Correspondence address: Guan Li-Ren, Department of Epidemiology; Shen Wei-Xia, Office of Scientific Service, Institute of Parasitic Diseases, Chinese Academy of Preventive Medicine, WHO Collaborating Centre for Malaria, Schistosomiasis and Filariasis, 207 Rui Jin Er Lu, Shanghai 200025, China.

EPIDEMIOLOGIC SURVEY

Epidemiological patterns of VL in China

Guan *et al* (1976) categorized endemic areas of VL in China into 3 types according to geographical landscape, age distribution of affected populations, species and ecology of vector sandflies and the role of canine leishmaniasis in epidemiology as well : The anthroponotic type is prevalent in plain regions, the anthropozoonotic type refers to the disease discovered in mountain/hill regions, and the enzootic type is that endemic in desert zones. The epidemiological characteristics of VL endemic in a particular desert area of Xinjiang was described in detail by Guan and Chai (1989) after making an extensive survey. The authors inferred that it might be a subtype of enzootic, which exhibited an epidemiologic pattern quite different from that of the general enzootic type. The specific features concern the following aspects: (1) The focus was usually localized in a stony desert region at the foot of mountains. (2) Ninety percent of the cases were children below 10 years of age, and adult cases were very few. (3) The transmitting vector is *Phlebotomus (Paraphleboto-*

mus) alexandri, an exophilic species, and it's natural infection rate for promastigotes of *Leishmania donovani* was rather high. (4) Patients could only be found in winter and spring of the next year, but could not be detected in the sandfly season. (5) To date, the dog was not evidently involved in this transmission cycle. This region is deemed to be a natural nidus of VL, with wild animals presumably being the source of infection. It is of interest to conduct further relevant investigations, especially on its distribution in northwest China.

VECTORS

The role of *Phlebotomus alexandri* in transmitting VL

According to the WHO criteria of 'Incrimination of vectors', Guan *et al* (1986a) pursued a study on *P. alexandri* in an endemic area of VL in the Turfan basin to the north of Tianshan mountains, Xinjiang. The results verified that this sandfly species is a local vector of VL. The finding obtained from Wensu county to the south of Tianshan mountains with the identical landscape further confirmed the role of *P. alexandri* in transmission of VL (Guan *et al,* 1986b). This was the first time a sandfly species of the *Paraphlebotomus* group had been reported to be a vector of VL in the eastern hemisphere.

The problem of *'Phlebotomus sichuanensis'*

Guan *et al* (1980a) initially found morphological diversity of *P. chinensis* distributed at different altitudes in south Gansu and north Sichuan. The male sandflies found below altitude 1600 m were generally smaller with the antennal formula 2/3-15 (Type A), whereas the males above altitude 1600 m were larger with the antennal formula 2/3-8 and 1/9-15 (Type B). Thereafter, Leng and Yin (1983) nominated Type B as a new species *'Phlebotomus sichuanensis'*, and indicated its role as a vector of VL based on the normal development in stomach and detection from pharynx of *Leishmania donovani* in this species of sandfly (Leng, 1988). After scrutinizing morphological characteristics of these two types of sandflies, including the pharynx, spermatheca and externalia, Xiong *et al* (1990) judged more recently that there were no fundamental differences between Type A and Type B. Apart from the fact that no consistent size difference was found between Type A and Type B, the antennal formula of the male sandflies would alter after 'cross fertilization' between the two types in the laboratory. Half of the G_1 males originating from G_0 males with antennal formula of 2/3-8 and 1/9-15 would change their antennal formula into 2/3-15, while no alteration was seen in G_1 from G_0 with the antennal formula 2/3-15. The conclusion was drawn that the minimal size differentiation between those two types of sandflies held no taxonomic significance, and *P. sichuanensis* should be the synonym of *P. chinensis,* hence elucidating this controversial problem.

Identification of promastigotes in naturally infected *P. major wui*

The result of dot and Southern hybridization with biotin labeled k-DNA prepared from *L. donovani* (isolated from a VL case in Sichuan Province) and IWUI/CN/77/BACHU 1 (isolated from an infected *P. major wui* in the Bachu desert area, Xinjiang) demonstrated marked homogeneity (Luo *et al,* 1989). The preceding results in association with the findings of Xiong *et al* (1974) demonstrated further that *P. major wui* is the vector of VL in the dry desert area of west China.

INVESTIGATION OF RESERVOIRS IN WILD ANIMALS

The reservoir of VL in wild animals in desert and mountain regions located at western part of China is still ambiguous. No positives were recorded after microscopic observation of hepatic and splenic smears from 1461 *Eutamias sibirious, 2 Felis bengalensis,* 8 *Meles meles* and 3 *Vulpes vulpes* (Li, 1988) in the mountainous region of Shanxi Province. Yin (1985) reported negative results from culture of hepatic and splenic tissues of 315 wild rodents which were caught in the north of Sichuan. However, Xiong and Jin (1989) detected promastigotes of *L. donovani* (Qu *et al,* 1990a) from naturally infected *P. chinensis* in wild caves in the mountainous region of Nanbing County, Sichuan Province. The deduction was thus made that sandfly infection *in situ* was from wild animal sources.

A total of 2183 wild rodents belonging to 9 genera and 12 species were successively autopsied to examine liver, spleen, lymph nodes, bone marrow smears and by culture by Guan *et al* (1984), using animal sources from Ejne banner, Inner Mongolia; by Zhao *et al* (1985) with animals from Bachu desert area of Xinjiang; and by Xu *et al* (1987) with animals from Turfan in Xinjiang *L.donovani* was not revealed in any case. Other wild animals were examined accordingly (Table 1) and bone marrow smears of 174 dogs were observed as well, all showing negative results. Guan *et al* (1984) detected amastigotes in blood monocytes of 9 out of 13 lizards *(Teratoscincus przewalskii)* from Ejne banner. Taking liver tissues from 5 infected lizards and subjecting these to NNN culture, promastigotes were present in all on the 15th day after cultivation, demonstrating that both direct tissue smear and *in vitro* cultivation are reliable and effective tools for detecting *Leishmania.* Seeing the fact that few VL patients could be found during July to August, the discovery of naturally infected sandflies then almost certainly implicated the existence of a sandfly-wild animal cycle *in situ.* Elucidation of whether wild animals could act as reservoirs of VL is important in respect of both disease control and research on evolution of visceral leishmaniasis. It would appear that carnivorous animals *(Canidae)* are the predominant ones to be considered.

THE PROBLEM OF GEOGRAPHICAL STRAINS

Owing to the different epidemiological patterns of VL in China (Guan *et al,* 1976), the idea of the existence of geographical strains of *L. donovani* has been framed. Commencing from late 1980s, Chinese investigators have pursued studies in this regard with advanced methodology and technology. Clear differentiation of *L. donovani* antigens of desert species origin from that of plain species origin was demonstrated by application of monoclonal antibodies (McAb) using dot-ELISA (Qu and Bao, 1987). Photobiotin labeled k-DNA was employed as a probe to hybridize with 10^3-10^8 promastigotes of different isolates of *L. donovani.* The results demonstrated that there were heterogeneities between isolates of *L. donovani* from patients in mountain and plain regions, whereas homogeneities were shown between those from mountain and desert regions (Luo *et al,* 1989). Guan *et al* (1990) conducted a study on the infectivity of *P. alexandri* (Xinjiang) of *L. donovani* isolated from the desert area of Xinjiang (A), the mountainous region of Gansu (B) and the plain region of Henan (C); infectivity was found to be high for isolate A, followed by isolate B, while low infectivity was revealed for isolate C. These data are consistent with the probable existence of diverse geographical strains of *L. donovani* with different

Table 1

Autopsy on wild animals in desert regions of west China.

Wild animals		No. autopsied		
		Dry desert area		Stony desert area
		Ejne banner[a]	Bachu county[b]	Turfan county[c]
Fox	*Vlupes vulpes*	113	1	0
Hedgehog	*Hemiechinus auritus*	78	33	4
Bat	*Plecotus auritus*	9	0	18
Cat	*Felis bieta*	10	0	0
Rabbit	*Lepus* sp.	9	11	4
Shrew	*Crocidura* sp.	0	0	9

a = Guan *et al,* 1984
b = Zhao *et al,* 1985
c = Xu *et al,* 1987

epidemiological patterns distributed respectively in plain, mountain and desert regions.

SANDFLY CONTROL

Experiments with 2.5% deltamethrin, 12.5-37.5 mg/m^2, indoor and outdoor spraying to control *P. chinensis longiductus,* a peri-domestic species of sandfly have been pursued in Kashen, Xinjiang (Chai *et al,* 1986). Subsequently, a drastic decline of sandfly numbers for 2 years resulted, but in the third year post-spraying a sandfly population revival to the same level as the control area was exhibited. The presumption was made that if all the patients in that locality could be cured in two years after deltamethrin spraying, VL control would be achieved. Another trial of deltamethrin spraying was carried out at a dosage of 25 mg/m^2 in the loess plateau in north Shaanxi for two consecutive years, 1985 and 1986 (Xiong and Jin, 1987). Marked decrease of peri-wild *P. chinensis* was observed in the day time.

Experiments on sandfly prevention indicated that DETA (N,N-diethyl-m-methyl-benzoylamide), domestically made 'Quwenlin' (a mixture of DETA and 3, 8-dihydroxy-p-menthane) and 'Wenpasui' (chemicals with amide groups) were promising repellents of sandflies when smeared on human skin at 0.25 μl/cm^2; the repelling time was 4-6 hours for *P. alexandri,* and 6-9 hours for *P. major wui.* The application of the above repellents will protect the susceptible residents and field-work staff in the endemic areas from sandfly biting, thus facilitating the interruption of VL transmission (Jia *et al,* 1989, 1990).

STUDIES OF CONTROL MEASURES

Control of infection source

Canine visceral leishmaniasis is the major infection source in mountain regions of south Gansu and north Sichuan. Infection rates of 7.6-14.0% were recorded among examined dogs upon examination of bone marrow smears (Li and Guan, 1990). The distribution of infected dogs could be as high as altitude 2080 m (Yin *et al,* 1990). Elimination of domestic dogs is an essential practice aimed at the prevalence of VL in local populations. A convincing example is the longitudinal study performed by the Gansu Institute of Endemic Diseases during 1976-1978. Resulting from extinction of 54.2-79.0% of domestic dogs annually in connection with medication of VL patients, the number of patients detected each year dropped from 177 in 1976 to 34 in 1978. Following the marked decline of dog numbers, the number of VL patients was around 8-14 in 1979-1982. Thereafter, the case number gradually rose with the increase of domestic dogs, leading to 28 cases in 1983, 120 in 1984 and 176 in 1985, resuming the original prevalence prior to canine extinction. Another dog elimination campaign combined with patient therapy was carried out by the Sichuan Institute of Parasitic Diseases in Nanping county, Sichuan province in 1976-1977, consequently no VL patient was detected during 1979-1982 (Li and Guan, 1990). The experience of the above two provinces showed that the effect of VL control was evident 4 years post canine extinction, provided the coverage attained 3/4 of the total number of dogs.

Insecticide spraying as major measure

Deltamethrin spraying in a patient's house and its neighbouring dwellings was implemented in combination with patient treatment in Wenxian country of Gansu province from 1985 to 1989. Continual but gradual decrease of patient numbers was evidenced as 176, 166, 158, 96 and 102 during these 5 years (Li and Guan, 1990). Inference is therefore drawn that in order to decrease the incidence of VL, it is preferable to introduce canine extinction rather than vector control.

SURVEILLANCE

Integrated intervention including free therapy using sodium stibogluconate and sandfly control with insecticides founded a solid basis to fight against VL and led to satisfactory results. Conspicuous achievements were presented by Shandong province where the prevalence of the disease had been as high as 3,500/million people in 1950, which dropped to 30/million people succeeding the control campaign in 1958, and further declined to 5/million people in 1963 and 0.1/million people in 1970. New cases have not been discovered there since 1972. *P. chinensis* disappeared in 84.8% of

the investigated villages, and was scarce in the others. When intradermal tests with leishmanin as antigen were performed on 5,285 children and young people below 20 years old during 1977-1988, no positive reaction was ever seen. The monitoring thus showed that the transmission of VL in Shandong Province has already been interrupted, and the transformation of a hyper-endemic area into a non-endemic one was the result (Shao and Wang, 1989). Taking Henan Province as another example, the prevalence of VL was 5000/million people in 1951, which decreased to 77/million people in 1958, and the disappearance of new cases has been recorded since 1973. A survey in late 1980s demonstrated that *P. chinensis* disappeared in the eastern plain region, whilst still present in the western hill region of the province (Yan *et al,* 1989). Only a few cases have been detected in north Jiangsu, north Anhui, south Hebei, north Hubei and the Guanzhong plain region of Shaanxi since the 1960s. As a whole, both leishmaniasis cases and *P. chinensis* diminished drastically or even disappeared in previously endemic areas, displaying consolidated achievements of disease control.

SPECIAL CASES

There were still a few adult cases of post-kala azar dermal leishmaniasis in the plain regions (Yu *et al,* 1983; Shao and Wang, 1989). In the mountain region of south Gansu, when two baby patients with VL (1.5 years old) received sodium stibogluconate at a total dose of 1.2g/kg body weight divided into 2-4 doses, pruritis with red papules from the size of a pin tip to that of a mung bean were present on the whole body after the therapy. *L. donovani* was shown in tissue smears taken from the rash. The two cases were cured with the disappearance of VL symptoms and dermal lesions after further administering sodium stibogluconate (1.8g/kg body weight) for 4-6 courses (Chen *et al,* 1988). It is the first report in China to document the dermal signs occurring during the medication period, and is noteworthy since misdiagnosis of eczema might be made.

Zhao *et al* (1988) carried out follow-up studies on dermal tissue of 245 VL patients post treatment in Kashen county of Xinjiang. Of 210 cases who had been cured less then 4 years before, 17 cases (8.1%) revealed *L. donovani* in dermal tissue taken from visually normal skin of scapular or lateral brachial sites, while no parasite was detected from those who had received treatment more than 4 years before. Thirteen out of the 17 cases with *L. donovani* in dermal tissues were followed up 4 years thereafter, the parasite was never found, indicating its disappearance from dermal tissue with the elapse of time. The significance of dermal parasite carriers in epidemiology remains to be clarified.

Zhang (1990) reported a rare case of VL in Xingping county of Shaanxi, who experienced recurrence of VL after long interval of cure for 26 years with sodium stibogluconate therapy. The relapse was evidenced by the presence of *L. donovani* in bone marrow smears.

IMMUNODIAGNOSIS AND IMMUNOLOGICAL STUDIES

It is not surprising to note that immunodiagnostic studies of VL have been relatively limited as compared with those in schistosomiasis and malaria, nevertheless promising attainments have occurred since late 70s. Antigen preparations of promastigotes were employed for VL diagnosis in a series of trials including direct hemagglutination tests (Chai *et al,* 1979), indirect fluorescent antibody tests (Chai *et al,* 1979; Guan *et al,* 1980b), indirect hemagglutination tests and counterelectrophoresis (Wang *et al,* 1981), enzyme linked immuno-sorbent assay (Qu *et al,* 1981) and FAST-ELISA (Wang *et al,* 1990a); the positive reaction rates fell into a range of 96.7-100%. With the merits of ease and quickness in operation, FAST-ELISA was considered most appropriate for field application. Awareness should be maintained while implementing field trials on ELISA and FAST-ELISA, that cross reaction (ca 10%) between leprosy and VL might occur.

Detection of circulating antigens from VL patients was performed by several laboratories. McAb-antigen dotting test (McAb-AST) was used to assess 101 VL patients and 509 sera from normal persons and individuals with other parasitoses and infectious diseases; a positive rate of 97.0% and a false positive rate of 0.2% were obtained (Hu *et al,* 1989). Qu *et al* (1990b) employed McAb in dot-ELISA to detect circulating

antigens in 159 VL cases, 144 (90.6%) showing positive reactions, while negative results were noted for 50 sera from normal persons and patients cured 3 months-16 years before. Using the same method, Xu *et al* (1990) tested 60 VL cases for circulating antigens in pilot areas of Gansu and Sichuan, the positive rate being 100%, while negative reactions were recorded for 23 patients cured 2 months to 4 years before, 119 normal persons and 106 cases of other infectious diseases (including leprosy), indicating that the McAb-dot-ELISA could be applied not only for diagnosis of VL but also for evaluation of therapeutic effect.

Immunological research concerning VL is far from profuse. A study on lymphocyte transformation showed that the natural lymphocyte transformation rates for VL patients (Group A), healthy persons with positive intradermal reaction using leishmanin as antigen (Group B) and healthy persons with negative intradermal reaction (Group C) were 2.18-11.31%; an increase to more than 80% was noted in all the 3 groups after adding PHA, while the rate was significantly higher in group A ($93.2 \pm 5.46\%$) than that ($81.3 \pm 9.82\%$) in group C ($p < 0.05$). Inhibition of lymphocyte response to PHA was exhibited with the concurrent presence of *L. donovani* antigens (Chai *et al,* 1981). Ultrastructural localization of *L. donovani* antigen by the immunogold labeling technique was studied. The antigen recognized by the protective McAb $2H_6$-E_3 was mainly localized on the outer side of the promastigote membrane; while that recognized by MaAb $1H_7$-C_2-E_7, was predominantly on the inner side of the promastigote membrane, but the outer side was also involved (Wang *et al,* 1990a, b).

HISTOCHEMICAL STUDIES

Some biochemical components of *L. donovani* were studied for their localization and content by histochemical techniques (Yang *et al,* 1987). The essential components of nucleus and kinotoplast were found to be DNA, RNA and protein bound α -amino groups; and alkaline protein, protein bound tyrosine, tryptophan and histidine were components of intermediate quantity; while glycogen was not detected. In contrast, there was profuse glycogen but minimal quantity of other components in the cytoplasm.

CONCLUSION

Great progress pertaining to epidemiology, vector species, *Leishmania* strains, immunodiagnosis and control measures of VL was made in recent years. On the basis of critical review of the previous work, recommendations have been put forward for carrying out research on some fundamental aspects. In view of the fact that VL has not been completely brought under control in mountain and desert regions in northwest China, efforts should be exerted on studying endemic factors, prevalence patterns, reservoirs in wild animals and the role of canine visceral leishmaniasis in the transmission of the disease. The advanced immunodiagnostic tools developed recently are expected to be helpful in elucidating animal reservoirs. Decrease of dog-sandfly contact in the mountain regions and of man-sandfly contact in the desert regions is important for the purpose of decreasing the incidence of VL in the local populations.

Having proved to be effective, sandfly repellents and insecticides are suggested to be used more widely, and formulation improvement should be taken into account so as to make them more acceptable for local inhabitants, hence their introduction to more extensive application in the field. The timely medication of new VL patients present in Kashen plain of Xinjiang as well as the further study on the significance of dermal *L. donovani* carriers in the prevalence of VL are proposed. It is of particular interest to explore intensively the deduced existence of different geographical strains of *L. donovani* for the sake of providing grounds for formulating more rational and effective control measures.

REFERENCES

Chai JJ, Li BS, Xu GZ, Zhang WL, Zhao EM. Studies on serodiagnostic method in kala azar. *J Epidemiol* 1979; 4 : 342-5.

Chai JJ, Li BS, Zhao EM. Lymphocyte transformation in kala azar. *Chin J Epidemiol* 1981; 2 : 251-2.

Chai JJ, Yimamu, Li JS, Li BS, Fu YF. A field trial of sandfly control with K-Othrine and 1-(3,4-dichlorophenyl)-2, 2, 2-trichloroethyl acetate. *Endemic Dis Bull* 1986; 1 : 64-9.

Chen SB, Wang XY, Liu PZ, Chen XW, Wei LS. Dermal lesion in two infant kala azar cases. *Chin J Dermatol* 1988; 21 : 319-20.

Guan LR, Wang J, Hu YD, Xiang GH, Zheng SC. Epidemiological patterns of kala azar in China and their relationship to control program. *J Contr Res Epidemic Dis* 1976; 3 : 225-32.

Guan LR, Wang J, Liu PZ, Zhang ZG. The bionomics of *Phlebotomus* in the mountainous regions southern Gansu and the Loess Plateau of northern Shaanxi. *Acta Entomol Sinica* 1980a; 23 : 25-31.

Guan LR, Wu KC, Xu YX, Jin CF, Chai JJ. Observation of the indirect fluorescent antibody test in kala azar with dried blood-filter paper method. *J Epidemiol* 1980b; 1 : 118-20.

Guan LR, Cao HX, Yu JG, *et al.* Investigation of kala azar hosts in Ejne banner, the Inner Mongolia Autonomous Region. *J Lanzhou Med Coll* 1984; 28 : 11-4.

Guan LR, Xu YX, Li BS, Dong J. The role of *Phlebotomus alexandri* Sinton 1928 in the transmission of kala azar. *Bull WHO* 1986a; 64 : 107-12.

Guan LR, Xu YX, Mao YD, Wang W. The sandfly fauna and its role in transmission of kala azar in four landscape zones of Aksu region, Xinjiang. *J Parasitol Parasit Dis* 1986b; 4 : 169-72.

Guan LR, Chai JJ. Kala azar in stony desert region of of Xinjiang, China. *Endemic Dis Bull* 1989; 4 : 40-4.

Guan LR, Xu YX, Wang G, Jia JX. Observation on the infectivity of *Leishmania donovani* from different areas to *Phlebotomus alexandri* from Xinjiang. *Chin J Parasitol Parasit Dis* 1990; 8 : 104-7.

Hu XS, Lin FQ, Liu Q, Kan B, Liu PN, Wang ZL. Further study on the application of McAb-AST to the detection of antigens from serum for diagnosis of kala-azar. *Chin J Parasit Dis Contr* 1989; 2 : 69-72.

Jia JX, Guan LR, Xu YX, Wang G, Zuo XP, Hao KF. Efficacy of 4 repellents against *Phlebotomus major wui. Pest Control* 1989; 5 [Suppl] : 131.

Jia JX, Guan LR, Xu YX, Wang G, Hao KF. Studies on the efficacy of five repellents against *Phlebotomus alexandri. Clin J Parasitol Dis* 1990; 8 : 203-6.

Leng YJ, Yin ZC. The taxonomy of *Phlebotomine* sandflies (Diptera: *Psychodidae*) of Sichuan Province, China, with descriptions of two species *Phlebotomus (Adlerius) sichuanensis* sp. n, and *Sergentomyia (Neophlebotomus zhengjiani* sp. n, *Ann Trop Med Parasitol* 1983; 77 : 421-31.

Leng YJ. A review of leishmaniasis research in China from 1960-1986. *Jpn J Parasitol* 1988; 37 : 119-32.

Li TX. 1975-1984 report on the surveillance of kala azar in Shanxi province. *Chin J Epidemiol* 1988; 9 : 294-5.

Li YM, Guan LR. Investigations of kala azar in the mountainous regions of Gansu and Sichuan provinces. *Clin J Parasit Dis Contr* 1990; 3 : 13-6.

Luo P, Hu XS, Xiong GH, Jin CF. Identification of *Leishmania donovani* isolates from different kala azar foci of China by k-DNA hybridization with biotin k-DNA probe. *Endemic Dis Bull* 1989; 4 : 61-5.

Qu JQ, Bao YF, Wang J, Wu YD. The micro-ELISA technique in the serodiagnosis of visceral leishmaniasis. *Chin J Microbiol Immunol* 1981; 1 : 134-7.

Qu JQ, Bao YF. Dot-ELISA using monoclonal antibodies for identification of *Leishmania donovani. Chin Med J* 1987; 100 : 823-6.

Qu JQ, Xu YX, Guan LR, Bao YF. Identification of *Leishmania donovani* promastigotes from *Phlebotomus* by monoclonal antibody. *Chin J Parasitol Parasit Dis* 1990a; 8 : 274-6.

Qu JQ, Xu YX, Bao YF, Yang YT, Wang JY. A study on circulating antigen in visceral leishmaniasis using peroxidase labelled monoclonal antibody. *Endemic Dis Bull* 1990b; 5 : 55-8.

Shao QF, Wang CT. Surveillance of kala azar for 30 years after basic elimination of the disease in Shandong Province. *Chin J Parasitic Dis Contr* 1989; 2 [Suppl] : 1-4.

Wang J, Bao YF, Hu YD, *et al.* Comparison of indirect hemagglutination (IHA) with counter immunoelectrophoresis (CIEP) in sorodiagnosis for kala azar. *Acta Acad Med Sin.* 1981; 3 [Suppl 2]; 74-5.

Wang XZ, Li ST, Yin ZC, Zhou ZX. Development and application of an ELISA kit containing anti-human gamma chain McAb for rapid diagnosis of leishmaniasis. *Endemic Dis Bull* 1990a; 5 : 48-51.

Wang ZL, Hu XS, Wang YP, Yang ZM, Li GR. Ultrastructural localization of *Leishmania donovani* antigen by immunogold labeling technique. *Chin J Parasitol Parasit Dis* 1990b; 8 : 1-3.

Xiong GH, Guan LR, Guo YK. Study on vectors transmitting kala azar in Xinjiang. *J Contr Res Epidemic Dis* 1974; 4 : 327-34.

Xiong GH, Jin CF. Studies on deltamethrin in the control of peri-wild *Phlebotomus chinensis.* Chinese *J Parasitol Parasitic Dis* 1987; 5 : 176-9.

Xiong GH, Jin CF. Studies on the longitudinal distribution of sandfly *Phlebotomus chinensis* and its relation to kala azar in southern Gansu and northern Sichuan. *Endemic Dis Bull* 1989; 4 : 15-21.

Xiong GH, Jin CF, Hong YM. *et al.* Further study on the type of sandfly *Phlebotomus chinensis* in southern Gansu and northern Sichuan. *Endemic Dis Bull* 1990; 5 : 53-60.

Xu YX, Jia JX, Guan LR, Zuo XP, Wang G. Investigation on animal reservoirs of kala azar in Meiyaogou of Turfan county. *Endemic Dis Bull* 1987; 2 : 76-7.

Xu YX, Wang XZ, Li ST, Wang JY, Wang JT. A field demonstration of determining circulating antigens of kala azar by direct Dot-ELISA. *Endemic Dis Bull* 1990; 5 : 66.

Yang YQ, Yang HZ, Guan LR. Histochemical observations on three *Leishmania* species. *Acta Zool Sin.* 1987; 33 : 151-4.

Yan QY, Xu RY, Shang LY, Wang HL, Shi DY. 1983-1988 report on the surveillance of kala azar in Henan Province. *Endemic Dis Bull* 1989; 4 : 70-1.

Yin ZC. Epidemiology of kala azar in Sichuan province. *Sichuan J Parasitic Dis* 1985; 3 : 24-34.

Yin ZC, Wu YX, Liu PZ, Guan LR. Study on the vertical distribution of canine visceral leishmaniasis in Wenchuan and Nanping countries, Sichuan province. *Endemic Dis Bull* 1990; 5 : 25-40.

Yu BT, Wang DB, Wen ST, Li YS, Cao XW, Jiao MY. Post-kala azar dermal leishmaniasis a case report. *Nat Med J Clin* 1983; 63 : 762.

Zhang ZG. A case of kala azar relapsed after 26 years. *Chin J Parasitol Parasitic Dis* 1990; 8 : 287.

Zhao EM, Chai JJ, Li BS, Li JS, Yimamu, Jiao LE. Epidemiologic survey of kala azar in Bachu reclamation area, Xinjiang. *J Xinjiang Med Coll* 1985; 8 : 259-62.

Zhao EM, Bai RL, Li JS, Jiao LE, Yimamu. Detection of LD body in skin of kala azar cases after treatment. *Chin J Prev Med* 1988; 22 : 26.