EPIDEMIOLOGY OF THE LEISHMANIASES IN SOUTHWEST SAUDI ARABIA

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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

« أَمَّنْ هُوَ قَانِتٌ آنَاءَ اللَّيْلِ سَاجِدًا وَقَائِمًا يَحْذَرُ الْآخِرَةَ وَيَرْجُو رَحْمَةَ رَبِّهِ قُلْ هَلْ يَسْتَوِي الَّذِينَ يَعْلَمُونَ وَالَّذِينَ لَا يَعْلَمُونَ إِنَّمَا يَتَذَكَّرُ أُولُو الْأَلْبَابِ »

إهداء

إلى - والدي الحبيبين اللذين كانا ومازالا وراء كل نجاح تحقق على مسيرة ربع قرن من طلب العلم والمعرفة كان أحد ثمارها هذا البحث

- زوجتي الغالية لصبرها وكفاحها معي

- أبنائي وبناتي الأحبة الذين عانوا كثيراً من وعثاء السفر

- إخواني وأخواتي الأوفياء وكل صديق لما ساهموا به من دعم معنوي ومادي في سبيل إتمام هذا البحث

والله أسأل أن ينفعنا بما علمنا وأن يختم بالصالحات أعمالنا .. آمين

محمد علي سعيد زاهي حسن الزهراني
لندن / ربيع الأول ١٤٠٩ هـ
أكتوبر ١٩٨٨ م

ABSTRACT

Visceral (VL) and cutaneous leishmaniasis (CL) are public health problems in the southwest of Saudi Arabia. The causative parasites, the vectors and the possible animal reservoirs in that area were all unknown before this study began.

Because of the size of the area and to achieve the different aims of the study, a laboratory was established in Abha City which is in the centre of the study area, altitude approximately 2000 m.

More than 50 isolates from CL lesions, 17 from VL patients and 700 human filter paper blood samples were collected. Eighty nine feral dogs were captured in Kala-azar endemic areas and examined for *Leishmania*. More than 8,000 sandflies collected from fixed stations were examined and more than 1600 *Phlebotomus* females were dissected. Forty four human isolates from human CL lesions were typed by the isoenzyme technique which showed that *L.tropica* is responsible for CL in both lowlands (altitude about 450 - 700 m) and the highlands (altitude about 2000 m). Only one zymodeme (LON-63) was found in the isolates from the lowlands but, in the highlands, four zymodemes (LON-10, 71, 72 and 73) were found. Zymodemes LON-10 and LON-71 were also isolated from *Phlebotomus sergenti*, which has been shown clearly to be a major vector of *L.tropica* in the highlands. Animal susceptibility experiments showed that neither BALB/c mice nor the golden hamster were susceptible to *L.tropica*.

L.donovani sensu lato zymodeme LON-42 causes zoonotic infantile Kala-azar in areas at altitudes of up to 700m. Neither the vector nor the reservoir host were identified in spite of an active search for them. The limited sero-epidemiological survey using the ELISA procedure revealed a high frequency of antibodies in children in Al Baha province, much greater than was previously believed to exist.

Feral dogs in this area were found to be carriers of typical L.infantum, NOT the parasite found in man. The prevalence rate in dogs was high (19.3%). The dog's possible role in the epidemiology of Kala-azar in the study area is discussed.

The entomological studies revealed that six species of Phlebotomus exist in the study area, with Ph.sergenti as the dominant species in the highlands and Ph.bergeroti in the lowlands. Some species such as Ph.arabicus are limited to high altitudes (about 2000 m), and others such as Ph. alexandri to low altitudes (up to 700 m). Ph.orientalis was found mainly in the highlands but a few samples were collected from the lowlands. Sergentomyia species were abundant in all areas. The seasonal distribution based on a longitudinal study indicated that the population peak in both ecological areas (high and lowlands) occurs in July. The factors including the collection method and trap sites controlling the apparent seasonal distributions are discussed. Statistical data on the total cases of CL and VL reported in The Kingdom are presented and data from the

study area are compared with those from other areas such as the Eastern Province (where *L.major* is dominant) to give an overall picture of the leishmaniases throughout the country.

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CHAPTER I REVIEW OF THE LITERATURE

Part 1: Epidemiology of the Leishmaniases in the Old World.

1. Visceral Leishmaniasis

1.1 The parasite

In 1903 in India, Leishman discovered the parasite of visceral leishmaniasis (VL) of man, also called "kala-azar", and correctly related it to the trypanosomes; in the same year Donovan saw bodies identical to those seen by Leishman. Ross in 1903 named the parasite *Leishmania donovani*.

In 1908 Nicolle gave the name *Leishmania infantum* to the parasite causing infantile kala-azar, found in Mediterranean countries. The early classification was based on the geographical and clinical aspects of the disease. This classification soon became inadequate. Garnham (1971) suggested that the parasite behaviour in insects and in the vertebrate host provided a better method of classification. Lainson and Shaw (1972), in reviewing the taxonomic problem of the New World leishmaniases stressed that information on the biology, morphology and immunology of *Leishmania* remain prerequisites to any sensible classification.

Bray (1974) concentrated in his review on the geography and biological methods, rather than on clinico-pathology, and classified the Old World *Leishmania* as two species; the name *Leishmania infantum* was given to the parasite causing

infantile visceral leishmaniasis with various members of the Canidae as reservoirs and the name Leishmania donovani was given to visceral leishmaniasis or classical kala-azar and post kala-azar dermal leishmaniasis which affect mainly adults in India, Kenya and the Sudan.

Lysenko (1971) recommended using the subspecies name Leishmania donovani archibaldi for the parasite of African kala-azar, but Bray (1974) stated that "while I am sympathetic to the idea of giving the Sudan parasite separate status, this cannot include the Kenya parasite which is patently different". However, according to Chance et al. (1978) and Schnur et al. (1981), using simple biochemical methods, Ethiopian isolates of the Leishmania donovani complex were indistinguishable from those obtained from the Sudan, Kenya, Senegal or the Mediterranean basin where L.infantum is endemic. Subsequently more discriminating studies by Gramiccia et al. (1982), and Le Blancq and Peters (1986a) demonstrated heterogeneity in the parasites of the L.donovani-L.infantum complex. Lainson and Shaw (1987) discussed in detail the methods which are used in the taxonomy of the Leishmania parasites and they stated that "until further data are avalable on these African parasites and some biochemical variants recorded from Italy, the Eastern Pyrenees and Iraq, they can at most be referred to as other possible species within the donovani complex."

As a summary the taxonomy of causative parasites of VL is still disputed. There is general agreement for retaining

L.infantum as a separate species in the Mediterranean region and L.donovani in the Indian subcontinent. In other endemic foci of VL in different regions, such as Kenya, the Sudan and Iraq, the parasites possess differences from both L.donovani and L.infantum. Some workers tend to put them into separate species, while others prefer to classify them as profile (zymodeme "so and so") within the species complex. To me the most acceptable classification up to now in the Old World is the nomenclature which is based on clinico-geographical distribution, i.e.

i. - L.donovani sensu stricto in the Indian subcontinent.

ii - L.donovani sensu lato in East Africa.

iii - L.infantum in the Mediterranean basin.

This nomenclature, together with the London zymodeme numbers for the isolates given by Le Blancq and Peters (1986a), will be used in the text that follows.

1.2 The human host

1.2.1 Age distribution

In the geographical area where L.infantum exists, children are mainly affected and the disease is rare in adults. In an epidemiological analysis of human cases from Italy, 78.7% of cases were in the age group from 0 - 10 years, with the maximum in the age group from one to three

years (Bettini *et al*.,1981). In North Africa, 86% of the cases are infants (Dedet and Belzzoug, 1985). In East Africa where the disease trends and causative parasites are different from classical infantile kala-azar, Southgate and Oriedo (1962) in their epidemiological study in Kenya reported that 33.3% of the cases in the group were aged over 18 years, with only 8.76% in the age group 0-3 years.

Sukkar (1985) mentioned that more than 11401 cases were recorded in the kala-azar centre in Iraq from 1971 to 1982, and more than 75% of these cases were aged from one to two years. Daud and Rageh (1987) reported 53 cases of kala-azar in the Yemen during 21 months in the Taiz republic hospital, and 56% of these were under 5 years of age.

Visceral leishmaniasis is being increasingly reported from Saudi Arabia, the majority of cases coming from the South Western province. Between 1980 and 1985 a total of 346 cases were notified to the Ministry of Health (Peters and Al-Zahrani, 1987). Age distribution analysis shows that most cases are found in children. The nature of the parasite has been confirmed by isoenzyme typing as *Leishmania donovani sensu lato* zymodeme LON-42 (Peters *et al*., 1985). Further discussion about these isolates will follow in the next chapter.

Various factors to explain the preponderance of infants are postulated by different workers. These include natural resistance of adults (Adler, 1964) or a decrease of acquired immunity. Bray (1974) in discussing the problem stated that:

" it was not known if children were preferentially bitten by infected sandflies or whether older people are in some way better able to control the infection. The latter seems more likely as no one had noted any preferential feeding upon children by Phlebotomus ariasi or Phlebotomus major."

Bettini et al. (1981) attributed the increase in Italy in the older age group as being due to the lack of acquired immunity, but this was not confirmed by work done in an endemic area of Brazil by Badaro et al.(1986b).

Nutritional status may affect the age distribution in Brazil. Badaro et al. (1986b) found that 77% of patients with leishmaniasis were malnourished before developing the disease.

Bradley (1987) discussing the problem remained equivocal, stating that "nothing is known as yet of the formal human genetic basis of susceptibility to VL".

1.2.2. Sex

In general there are no significant differences between males and females in relation to infection with kala-azar (Bettini et al.,1981; Sukkar, 1985). However, Southgate (1977) in his study in Kenya, found that males were affected more than females, and explained that adult males are exposed to the vector by their custom ofsitting near termite hills.

1.2.3. Place of infection

The focality of the disease is determined by the parasite-vector-host interaction, and by environmental conditions. Lysenko (1971) described four types of foci of VL:-

i. Natural foci: Where the transmission occurs from animal to animal through the sandfly vector. Man is involved accidentally.

ii. Semi-synanthropic: Where transmission occurs between wild animals and domestic animals and man through the vector.

iii. Synanthropic: Where the dog is the main reservoir of infection and man is frequently involved.

iv. Endemic foci of Indian kala-azar: Where only man is involved.

Adler and Theodor (1931) stated that "house to house infections are not the rule, there is usually an interval of 30 meters between one case and another". The disease is commonest among the rural, the poorer rural or semi-rural populations."

Ho *et al*. (1982) in Kenya disagree with the termite hill hypothesis of Southgate and Oriedo (1962), as they found a marked homestead distribution of patients and the disease occurred in the homestead with or without being near the termite hills.

In the Sudan, Hoogstraal and Heyneman (1969) found that sandflies were biting man in the *Acacia-Balanites* forest

where *Phlebotomus orientalis* is abundant, but German workers in the Sudan (Zeese and Frank,1987) found that the *Acacia-Balanites* forest was not an essential habitat for transmission. Other examples of the focality of the disease were reported by Bettini *et al*. (1981) who found a higher incidence of cases occurring in restricted areas.

In Saudi Arabia the ecology and epidemiology of VL has not been discussed in a published report, although the case reports and the Ministry of Health reports have revealed that all the cases originated in the lowlands of the southwest of the country. From my experience in the field, these cases come from rural and semi-rural areas in the lowlands which are characterised by the hot climate and the clay soil. More discussion on this subject will follow in the next chapter.

1.2.4. Sub-clinical infection

The existence of subclinical infection is very important and very significant in the epidemiology of the disease. It is the "tip of the iceberg" phenomenon (Garnham, 1977).

Asymptomatic or cryptic infection, and/or subclinical infection do exist and are reported from different endemic areas. The ratio of asymptomatic or subclinical infections are reported to be 6:1 in Italy (Pampiglione *et al.,* 1974; and Bettini *et al*., 1980), 5:1 in Kenya (Ho *et al*.,1982) and 6.5:1 in Brazil (Badaro *et al*.,1986b).

Controlled longitudinal studies which were carried out

in Brazil by Badaro et al.(1986c) showed that among 86 asymptomatic children seen in the beginning of the study, twenty (20) remained asymptomatic during the follow-up, where another fifteen (15) developed classical signs and symptoms of kala-azar. The remaining children, 51 (59.3% of the total examined) had subclinical forms, while 13 out of 51 (25%) progressed to classic kala-azar with long incubation periods. The other 38 remained in a subclinical form for an average of 35 months before they recovered.

The prevalence of clinical and subclinical VL may be due to genetic variation or variable inoculation of parasites (Bradley, 1987). Other factors, age and nutritional status at the time of infection may contribute to this (Badaro et al. (1986b). Pampiglione et al. (1974) discussing the role of cryptic infection, stated that "cryptic infections could also be responsible for those cases of post kala-azar dermal leishmanoid which occur in India where there is no previous history of treatment for VL. Such cryptic cases could form reservoirs for future outbreaks when environmental conditions were suitable as in the case of Brill's disease in epidemic typhus".

In general, the epidemiological importance of both asymptomatic and subclinical phenomena are not clear and still equivocal.

1.2.5. AIDS and kala-azar

Pancytopenia is one of the major signs in kala-azar

patients. The mechanism of that is not fully understood as yet. One of the explanations is that it may have an immunological aetiology (Woodruff, 1973). However, in 1973 the AIDS was not yet recognized. An immunological mechanism was supported by Bada (1979) when reporting asymptomatic cases of kala-azar without pancytopenia. He found that "enlarged spleen plays a most important role in the pathogenesis of pancytopenia in kala-azar", but left the question open of whether hypersplenism acts only through immunological mechanisms or not.

Pancytopenia occurring in patients with AIDS has now been reported by several workers. Clauvel et al. (1986) reported kala-azar in an AIDS patient, the symptoms having developed by the patient's second visit after 5 months. At the first visit, a serological test by IFAT was negative, but bone marrow aspiration revealed Leishmania organisms. The patient probably acquired the infection while in southern France in April and June 1985. The author suggests that a diligent search for visceral leishmaniasis is necessary when pancytopenia occurs in patients with AIDS who have spent time in an endemic foci for leishmaniasis, even if they do not show enlarged liver and/or spleen.

Antunes et al. (1987) reported a case of VL in an AIDS patient who later on had a relapse. They called attention to "not only the importance of VL as a possible infection in patients living or travelling through endemic areas, but also the possibility of recrudescence in these immuno-

compromised patients."

1.3 Animal reservoirs

1.3.1. Dogs as primary host

In the identification of reservoir hosts the following criteria should be applied (Killick-Kendrick and Ward, 1981).

(i) The parasite isolates from suspected reservoirs are indistinguishable from isolates from man.

(ii) Infections are not rare in the population of the animal.

(iii) The vector feeds readily and frequently on the suspected animal.

(iv) The course of infection of the animal is such that parasites are easily picked up by feeding sandflies.

In practical terms, none of the animals (vertebrate or invertebrate) meet all these criteria.

Since Nicolle and Comte (1908) discovered the dog infected with Leishmania infantum in Tunis, many workers have accepted this discovery, and extensive work has been carried out on epidemiology and pathology of the infection. The early thoughts that the dog was an important reservoir were based on three criteria; first, the abundance of dogs in endemic areas which share man's habitats; second, the high prevalence of the disease in dogs; and the third was based on the early work of Adler (1936) when he showed skin

infection in the dog. He stated that "the skin infection is of varying intensity but it is always practically uniform and no parts escape. There is a tendency in the proliferating macrophages both parasitized and non-parasitized to localize themselves around hair follicles and sebaceous glands, and this phenomenon accounts for seborrhoea and partial depilation which has been so frequently noted. The obvious importance of skin infection lies in the fact that arthropods can feed on dogs with sufficient skin parasites."

Infected dogs have been reported from different countries where the Leishmania infantum exists. In Europe, French workers in their series of studies of human and canine visceral leishmaniasis, showed that the disease still exists in that country. Lanotte et al. (1978) reported a 4.46% infection rate among dogs in the Cevenne district. In Italy, 37% of dogs were serologically positive for visceral leishmaniasis (Mansueto et al., 1982).

In North Africa, infection rates of dogs were 6.2% and 11.4% in Tunisia and Algeria respectively (Dedet and Belazzoug, 1985). In Morocco, human and canine leishmaniasis were reported by Kirmse et al.(1987). In East Africa, one dog has been found infected in the Rift Valley (Ngoka et al., 1977), but this individual finding was not supported by other workers. In the Sudan, the NAMRO-3 team failed to find any infected dogs in the endemic area in spite of the high prevalence in the local inhabitants (Hoogstraal and

Heyneman, 1969).

In contrast to the situation in East Africa, the dogs have been found infected in Senegal without any recorded human cases (Ranque, 1977).

In Iraq, in spite of extensive studies for the reservoir, there are few reports of positive findings. A positive isolate in a pack of foxhounds near Baghdad has been reported by Sheriff (1957), and one isolate from the spleen of a dog from an endemic focus in the central region (Sukkar, 1981). In an extensive study in Baghdad City and neighbouring areas, Naizi (1980) examined more than 65 dogs. No parasites could be isolated, but the seropositivity rate was 10.8%.

In Saudi Arabia, no prior field study had been carried out in the kala-azar area, but our own observations discussed later on in Chapter V. High positivity rates of infected dogs have been found, and also successful isolates have been obtained and typed isoenzymatically.

At this stage, I would like to emphasize different points about dogs as hosts or vertebrate reservoirs:

i. The advent of isoenzyme typing which helps to match the dog isolates with human isolates, has shown that they are identical in some areas (Le Blancq and Peters,1986a), whilst in other areas this has not been confirmed.

ii. The percentage of infected dogs reported by different workers is subjective and depends on the diagnostic methods used. This point should be taken into account when

comparing the prevalence data (Ashford and Bettini,1987). A seasonal variation in canine VL has been reported and factor may affect the results.

1.3.2. Other possible reservoirs

This review will cover other possible reservoirs, either in the region where Leishmania infantum exists and/or regions where Leishmania donovani sensu lato exists.

Bray (1974), in his review of the reservoirs of L. infantum, stated that "the true home of L.infantum is probably the fox, jackal, wolf and porcupine. It seems probable that dog first acquired the infection from such wild sources".

In the Cevennes, France, the fox, Vulpes vulpes has been incriminated as a natural host of L.infantum (Rioux et al.,1968; Rioux et al., 1971b; Lanotte et al., 1970, cited by Ashford and Bettini, 1987).

In Portugal four foxes were positive and the parasites were found to be identical to other isolates in Portugal from man and dogs, (Abranches et al., 1984).

In Italy, the parasite isolated from one fox, Vulpes vulpes (Bettini et al., 1980), varied from human isolates in four enzymes (Gramiccia, 1982). Four isolates from Rattus rattus have been obtained from Italy (Bettini et al., 1980; Pozioet al., 1981). The isoenzyme typing of these isolates was indistinguishable from human visceral leishmaniasis. Further work by Gradoni et al. (1983) was carried out on

experimentally infected R.rattus. This species is now considered to be a natural reservoir of Leishmania infantum in Tuscany, Italy (Ashford and Bettini,1987).

In the Sudan, the rodents, Arvicanthis niloticus, Acomys albigena and Rattus rattus and two Carnivores, Genetta senegalensis and Felis serval were found to be infected (Hoogstraal and Heynemann, 1969). The Arvicanthis isolates were identical to some human isolates, (Zymodeme LON-48, Le Blancq and Peters, 1986). Recent reports from the Sudan indicated that, in spite of examining more than 1,600 small mammals of several species, no parasite could be detected in any of them (Zeese and Frank, 1987).

Infected Arvicanthis niloticus has been reported from Ethiopia and 7 isolates out of 117 examined were infected. One of the isolates was different biochemically from the parasites of VL from the same locality (Haile and Lemma, 1977; Chance et al, 1978).

In Kenya, the rodent isolates from Baringo, north Kenya were proven not to be identical with human visceral isolates (Chance et al., 1978), and the implication of any animal species as reservoirs is still equivocal.

Man-to-man transmission in African regions is postulated by different workers, among them Ho et al.(1982), and Ashford and Bettini (1987) who stated that "the question of man's role in the maintenance of kala-azar in East Africa has not been settled. It is generally supposed that sporadic cases in the Sudan and north Kenya are of zoonotic origin

but that, during epidemics in the Sudan and Central Kenya, man-to-man transmission takes place of VL, as is well illustrated by the history of the infection in Kenya.

In Iraq more than 243 jackals, 511 rodents and 114 foxes were examined (Bray and Dabbagh, 1968; Sukkar, 1978; Kadim et al., 1979; Niaz, 1980). No Leishmania parasites were isolated from any of these animals but in the jackals the seropositivity rate was 60%, with high titres of (up to 1/128 by IFAT) among them, compared to the low positivity rate (10%) in foxes and dogs (Niaz,1980).

In parts of Saudi Arabia where kala-azar is endemic, nothing is known of possible reservoirs, either in dogs or other animals. The VL cases in humans came from small villages where the fox, wolf, jackal and various rodents exist.

1.4. The vectors

The distribution of the vector is principally governed by climate, soil type, vegetation, altitude and the availability of suitable vertebrate hosts.

According to Killick-Kendrick and Ward (1981), five degrees of certainty govern the incrimination of a sandfly species as a vector. These degrees are:

Grade 1. Anthropophilic species of sandflies are present in areas where man becomes infected.

Grade 2. The distribution of suspected vectors should accord with the distribution of the disease in man and sandflies

should be sufficiently abundant to assume that they maintain the transmission of the parasite in nature.

Grade 3. Leishmania should be isolated from wild-caught sandflies and be shown to be indistinguishable from parasites causing disease in man in the same place.

Grade 4. The development of the parasite occurs in naturally infected flies, or flies permitted to engorge on infected vertebrates and then maintained in the laboratory.

Grade 5. Experimental transmission of Leishmania by bite is generally considered as conclusive proof that sandflies are vectors of given parasites.

Many workers in the field from different regions over all the world have reported different species in the genus Phlebotomus as vectors of VL. Few of them have met all the criteria to be considered as a proven vector.

In the Cevennes, France, P.ariasi is the proven vector by all these criteria (Killick-Kendrick and Rioux, 1981), although P.perniciosus is a vector in southeastern France. (Dedet and Belazzoug, 1985) discussing the vectors which exist in North Africa, mentioned strong evidence that P. perniciosus is the main vector of VL in North Africa, with Ph.longicuspis as a secondary vector.

P.orientalis was said to be the vector in the Sudan (Hoogstraal and Heyneman, 1969). The promastigotes have been isolated from this species and proved to be identical to the isolates from human VL (Chance et al., 1978). In Kenya, P.martini was assumed to be the vector (Minter et

al., 1962). Also, in the north of the country, P.orientalis may be a vector (Ashford and Bettini, 1987).

In Ethiopia, it was assumed that P.orientalis is the vector in the Humera area (Fuller et al., 1979). Ashford and Bettini (1987) stated that "African VL is thought to be closely allied to the distribution of the two specific vectors, P.orientalis in the north and P.martini, further south".

In Iraq, P.papatasi and rarely P.alexandri were collected from houses of patients in the central region (Sukkar, 1985). According to the criteria mentioned by Killick-Kendrick and Ward (1981), this evidence was inconclusive.

In Saudi Arabia, Lewis and Büttiker (1982, 1986) reported 25 species of sandflies, ten (10) species are from the genus Phlebotomus, and among them six species are reported from the southwest where kala-azar is endemic. These are P. bergereoti, P.papatasi, P.alexandri, P.saevus, P.orientalis and P.nagbenius. One of the objectives of my study was to try to identify the vector(s) of VL and more discussion will take place in the next chapter.

1.5 Kala-azar in Saudi Arabia

Unconfirmed kala-azar was reported from a patient who had lived for fifteen years in Mecca (Phillips, 1904). The first confirmed local case of kala-azar in Saudi Arabia (from Qatif oasis) was reported in 1953 by Tarrizzo et al. More cases have been reported by Chowdhary (1976) and El-

Behairy et al. (1982). In 1983, an NLRP team (unpublished report) examined 66 school children in Gizan, 42 of whom had enlarged livers and spleen. 6 children out of these 42 were positive for antibodies of kala-azar. The Ministry of Health, represented by the Leishmania Unit, reported 36, 91, 188 and 248 cases in 1983, 1984 and 1985 and 1986 respectively (see Table 1). No precise epidemiological investigation has been made for these cases, but most of them have been reported as originating from Gizan, Abha and Al-Baha Province. Transmission may also occur sporadically in other parts of the Kingdom although this has never been proven.

Age distribution studies of kala-azar show that cases are mostly in children, which indicates high endemicity. Reports from investigators of NLRP and WHO (unpublished reports, 1983) indicated that the prevalence of kala-azar is greater than the reported cases suggest. Reasons for an apparently low prevalence are (a) the absence of diagnostic facilities for precise diagnosis, especially in areas where malaria and schistosomiasis are endemic, and (b) lack of awareness by physicians with no experience of the disease.

The parasite causing kala-azar in Saudi Arabia has not been well documented. Helpful information concerning the causative organism has been provided in Peters et al.(1985), in which two cases of kala-azar were identified by isoenzyme typing, and designated as L.donovani sensu lato, LON-42. The parasite is identical to the zymodeme of L.donovani which

TABLE 1 Regional distribution of kala-azar in Saudi Arabia (1983-1986) (Source: Ministry of Health, *Leishmania* annual reports)

Province	Year	Sex		Nationality		Age distribution					Total
		M	F	Saudi	Other	<1	1-5	5-15	15-45	>45	
Gizan	1983	9	6	13	2	-	-	-	-	-	15
	1984	24	19	31	12	0	31	12	0	0	43
	1985	55	35	79	11	15	58	17	0	0	90
	1986	125	57	132	50	22	114	38	6	2	182
Asir	1983	4	6	10	0	0	0	8	2	0	10
	1984	22	19	35	6	1	30	10	0	0	41
	1985	52	46	89	9	4	61	22	6	5	98
	1986	32	29	55	6	1	50	5	5	0	61
Al-Baha	1983	7	1	8	0	0	5	3	0	0	8
	1984	3	1	4	0	0	4	0	0	0	4
	1985	0	0	0	0	0	0	0	0	0	0
	1986	0	2	2	0	0	2	0	0	0	2
Western (Mecca, Taif, Jeddah)	1983	0	3	0	3	0	0	3	0	0	3
	1984	3	0	2	1	0	3	0	0	0	3
	1985	0	0	0	0	0	0	0	0	0	0
	1986	1	2	3	0	1	1	0	1	0	3

occurs in the east of Ethiopia, but differs from typical *L.donovani* in India and from typical *L.infantum* in Tunisia. Full characterization of as many cases as possible would give a better idea of the structure of the focus of disease, which will help with its control.

2. Cutaneous Leishmaniasis

2.1 Prevalence and distribution of the disease in Saudi Arabia

In 1971 the Central Public Health Laboratory reported 32 cases of cutaneous leishmaniasis (Morsy and Shoura, 1976). In Riyadh districts 273 cases have been reported by the same authors. In a survey carried out in Asir Province (low and high land) a total of 736 people were examined, from which 13 proven cases of cutaneous leishmaniasis were found, with 11 out of 13 cases being from the lowlands (Tihama), (Büttiker *et al*. 1982). The disease has been reported from Bisha, a town in the south-west of Saudi Arabia (Sebai and Morsy, 1976) where 110 cases were found among 620 out-patients of Bisha Hospital in one month. ARAMCO Health Department (1980) reported that between 1960 and 1969 only 7 cases were found in their medical centres, whereas in the subsequent 10 years (1970-1979) a total of 548 cases were treated, and the majority of patients were autochthonous cases from Al-Ahsa oasis (Al-dafas and Mohammed 1985). An epidemic found in foreign workers in Hofuf has been reported by Bienzle *et al*. (1978). The problem of leishmaniasis and

its distribution in the Kingdom has been further documented in a review by Nadim et al. (1979c). In 1980, a Leishmaniasis Unit was set up by the Ministry of Health and established an obligatory reporting system for cutaneous and visceral leishmaniasis in all health departments in each province in the Kingdom.

Table 2 shows the case distribution according to each province from 1978 to 1985. I should mention the following points from this table:

i. The increase in cases was a natural result of activation of the case diagnosis and reporting. The cases were found through the dermatology out-patients clinic of hospitals and primary health care centres.

ii. The National Leishmaniasis Research Program (NLRP) was established in 1982 at King Faisal University, which was to stimulate the interest of all workers in the health field.

iii. Increased man-fly contact by disturbing ecology of natural cycle between vectors and reservoirs.

iv. Around 40% of the diagnosed CL cases were in children under the age of 15 years. Sebai and Morsey (1976) reported 58 cases of children out of 110 total cases in Bisha. Al-Gindan et al.(1983) reported a first case of mucocutaneous leishmaniasis in a 5-month-old Saudi infant, a case identified as L.major by using biochemical typing. Another case of cutaneous leishmaniasis in a 5-month-old patient

has been reported from the Riyadh district by Büttiker *et al.*(1980).

v. Multiple lesions have been seen in many of the reported cases.

vi. Much of the diagnosis of cutaneous leishmaniasis depends upon the clinical basis, few cases being confirmed by direct smears for microscopical examination. Neither culture nor biochemical differentiation has been made, therefore determining the degree of prevalence and distribution of *L.tropica* and *L.major* has been speculative.

vii. A general survey in Saudi Arabia, which will give a better understanding of the prevalence and distribution of disease, has been carried out by Peters *et al.*(1985). In this survey 75 isolates of *Leishmania* were identified from different areas using isoenzyme typing, a technique considered to be the best method for determining and differentiating the different species of *Leishmania*, and the variant of each species. *L.major* zymodeme LON-4 has been found in 52 out of 57 isolates of *Leishmania* taken from man, animals and sandflies; one of these isolates has been identified as a new variant of *L.major* (zymodeme 65). *L.tropica* was found in two isolates, which led the authors to speculate that *L.major* is the most widely distributed species throughout the Kingdom.

TABLE 2 Cases of cutaneous leishmaniasis reported in Saudi Arabia (1978-1987).

Region	Year								
	1978	1979	1980	1981	1982	1983	1984	1985	1986
Al-Baha	-	-	-	-	-	395	1297	3062	1596
Central	92	130	235	445	618	4871	3127	2364	4501
Eastern	701	579	1277	1049	1318	1646	1743	1663	4382
Al Gisum	117	1239	1007	1225	2037	6047	3135	2013	1464
Hail	84	158	298	215	154	3533	970	567	1026
Almidena and Tabuk	0	244	506	249	543	895	877	1216	1322
Asir	0	33	69	88	299	810	1293	2144	1989
Gizan	-	-	-	-	-	8	0	85	97
Najrin	-	-	-	-	-	41	44	38	56
Western Province	49	86	129	246	414	33	52	18	170
Northern Province	0	0	0	0	0	39	39	15	8
Total	1043	2469	3521	3517	5383	18318	12577	13185	16611

viii. L.tropica seems to exist in mountainous areas such as Al-Baha, Taif and Asir. Nadim et al. (1979c) reported P.papatasi from one location only in Taif. Marinkelle (1980, unpublished report) did not observe any mammal burrows belonging to known rodent species usually involved as reservoir hosts of L.major. Also he observed that the lesions in man were usually single and that children under five years of age were more affected.

ix. Leishmaniasis reported from Mecca Province is low compared with other provinces, with 33, 52 and 18 cases reported in 1983, 1984 and 1985 respectively. Nadim et al.(1979c) suggested that P.bergeroti may be the vector. P.papatasi was not reported, but until now there has been no extensive vector survey in this area. In 1985a Peters reported the possibility that other types of Leishmania may be introduced from other parts of the world by pilgrims, who stay longer in Medina Al-Minoura than in Mecca; the ecology of Al-Medina is such that it is more suitable than Mecca for disease incidence, which means both areas should be studied and protected from Leishmania transmission.

2.2 Leishmania tropica

2.2.1 The parasite

The etiological agent of urban cutaneous

leishmaniasis (UCL) or some workers call it, anthroponotic CL (ACL).

It is taxonomically a separate species from L.major. The etiological agent of (UCL) has now been confirmed by the isoenzyme technique (Le Blancq and Peters, 1986b).

L.tropica itself showed heterogeneity in its isoenzyme profile and more than 18 (London) zymodemes have been reported from widely separated geographical areas.

L.infantum may cause CL similar to that caused by L.tropica, and vice versa L.tropica can visceralize (Schnur et al., 1981; Rioux et al., 1985). In Saudi Arabia, L.tropica exists in mountainous areas and in the southwest, two isolates from patients who lived in this area were identified as L.tropica (Peters et al., 1985).

2.2.2. The reservoir

Man was considered to be the main reservoir for Leishmania tropica (WHO, 1984). However, dogs have been found infected in endemic areas in Russia (Lysenko, 1971). In Afghanistan, Nadim et al.(1979b) found that 5 out of 197 dogs examined were infected with sores clinically resembling the sore in man. No parasite has been isolated or typed from this study. It seems unlikely that dogs are a main reservoir but that their role is secondary (Bray 1974; Nadim et al., 1979b). Pringle (1957) assumed that dogs played no significant part as reservoirs of L.tropica in Iraq.

L.tropica has been isolated from viscera of Rattus rattus

in the Baghdad Area, Iraq (El-Adhami, 1976; Al-Jabor et al., 1980). However, in spite of the failure to find an animal reservoir, some workers believe that a natural animal reservoir may exist. Bray (1974) stated "I feel inclined to believe that an urban reservoir is still to be found despite the negative evidence".

2.2.3 The vector

Several species of the genus Phlebotomus have been speculated to be vectors of L.tropica, among those which are strongly suspected is P.sergenti. The distribution of P.sergenti is in Central Asia, southwest Asia, India, North Africa and Southern Europe (Perfiliev, 1968; Lewis, 1982).

In Saudi Arabia P.sergenti is distributed in the high land southwest of Saudi Arabia, at an altitude of about 2000 metres above sea level (Büttiker et al., 1982; Lewis and Büttiker, 1986; Nadim et al.,1979c; Al-Zahrani et al., 1988).

The role of P.sergenti in transmission of L.tropica has been reported from USSR, Afghanistan, Iran and Iraq. In the epidemiological study in Afghanistan, Nadim et al.(1979b) stated that "on the basis of high density of P.sergenti in all infected areas, finding one case of natural leptomonad infection in this sandfly and absence of P.papatasi from some of the infected villages in the mountainous area, it seems that P.sergenti is the most probable vector of this type of disease in Afghanistan".

In the Baghdad area of Iraq, Adler and Theodor (1929) found that the human cases of oriental sore were associated with the distribution of P.sergenti. They also reported that 2 females out of 683 were found infected with promastigotes. Obviously the differentiation between L.tropica and L.major by isoenzymes was not known at that time, which left the identity of this isolate obscure.

The identity of the parasite found in P.sergenti was reported for the first time by Al-Zahrani et al. (1988) from the southwest of Saudi Arabia where they found that the isolates from two infected females were identical to human isolates of L.tropica from the same area by using isoenzyme typing (see Annex 17).

2.3 Leishmania major

2.3.1 The parasite

The separation of L.major as a separate taxonomy was supported by isoenzyme typing (Le Blancq et al 1986), and at least four zymodemes (London) are recognized from different geographical areas in the Old World.

In Saudi Arabia L.major LON-4 has been isolated from patients in an endemic area in Al-Ahsa Province (Peters et al., 1985) and from wild animals (Elbihari et al. 1987). Moreover, a parasite new to science, L.arabica, was isolated from 4 Psammomys obesus, and one feral dog from the same area. L.arabica, unlike L.major, has never so far been

found in man (Peters et al. 1986).

L.major LON-4 has been identified in other countries, Kuwait (Al-Taqi and Evans, 1978) Iraq (Al-Jeboori and Evans, 1980, and Yemen (Rioux et al. 1986).

The parasite belonging to this zymodeme (L.major LON-4) would seem to exhibit certain characteristics peculiar to it:

(1) The distinct pathology in the ear of Psammomys obesus (Elbihari et al., 1987).

(2) Unlike L.major from north and west Africa, L.major LON-4 zymodeme 4 has never been isolated from the viscera of reservoir animals (Killick-Kendrick et al. 1985; Elbihari et al 1987).

2.3.2 The reservoir

The animal reservoir of zoonotic cutaneous leishmaniasis (ZCL) has been described in detail, and meet all the criteria of reservoirs (Killick-Kendrick and Ward, 1981; WHO 1984) in Soviet Central Asia, which clearly shows that Rhombomys opimus is the maintenance host and the main reservoir of human infection (Petrisceva, 1971). Psammomys obesus and Meriones crassus have been found in the Negev desert (Schlein et al, 1984).

In Saudi Arabia, several investigators have tried to investigate the animal reservoir in Saudi Arabia. Morsy and Shoura (1976) in Al-Kharj, near Riyadh, examined a variety of animals (45 dogs, 15 cats, 12 foxes, 6 dab lizards, 3

hedgehogs, 9 weasels and 187 rodents) and they found amastigotes only in one dog and in two unidentified gerbils. No culture was made and no parasite identification was done. Therefore this finding still leaves open the question of the parasite identity and possible role of the animals as reservoirs for human infection. Büttiker and Lewis (1979), by analogy with the epidemiology of CL in countries with similar ecology to the Eastern Province, speculated that Meriones crassus and M.libycus syricus may serve as reservoirs in Al-Ahsa area in the Eastern Province, but they stated that Psammomys obesus, a well known reservoir of L.major in other countries of North Africa and the Middle East, was absent from there.

The first positive identification of animal infections with Leishmania major were those of Elbihari et al. (1985) where they succeeded in trapping 15 Ps.obesus in the Al-Ahsa area and they isolated the parasites from 6 of them. None of the 20 M.crassus nor the 2 Mus musculus examined by Elbihari proved to be postive. Subsequently L.major LON-4 was isolated from a total of 15 Ps.obesus, one M.libycus and one feral dog from Al-Ahsa area (Peters et al, 1985).

The infection rate among the Ps.obesus in Al-Ahsa area is exceptionally high. 186 (93%) out of 202 examined had lesions (Elbihari et al, 1987). The pathology of infection in Ps.obesus in Al-Ahsa was described by Elbihari et al. (1987), including the gross appearance of the lesion in the ears where the organism occurs. In only one instance was a lesion found on the tail. All other infected Psammomys had

ear lesions only. The author stated that "the lesion first became apparent as a slight swelling which in further development resulted in rounding of the edges of the ear". They reported that hyperpigmentation occurred with swelling of ear. As mentioned in section 2.3.1, the extensive study of ZCL also led to the discovery of a parasite new to science, L.arabica. In conclusion, in the Eastern part of Saudi Arabia, Leishmania major LON-4 is isolated from man and Ps.obesus, which is proven by all criteria to be the main reservoir of infection in the Al-Ahsa area. One point more about the animal reservoir is that the nature of the zoonotic source has not always been detected, and the human infection has been the only indication of the presence of the parasite (Ashford and Bettini, 1987).

2.3.3 The vector

Among the few species of the genus Phlebotomus which is proven to be a vector for Leishmania major is P.papatasi. This species has a wide range of distribution, is wholly domestic and an aggressive man biter (Lewis and Ward, 1987). The role of P.papatasi in transmission of Leishmania major has been best elucidated and explained by the Soviet workers.

Discussing the relationship between sandflies and Rhombomys opimus, Ashford and Bettini (1987) stated that "the burrow system provides protection against extremes of external temperature and desiccation, and harbours considerable organic debris. This is exploited by a wide

range of animals including sandflies. Although despite extensive searching, sandfly larvae have rarely been found in R.opimus burrows, emergence traps and other methods clearly indicate that these are important breeding sites for P.papatasi, P.caucasicus sensu lato and S.arpaklensis. The first two forms are important vectors of Leishmania, while S.arpaklensis transmits lizard leishmaniasis in the same habitat".

In Saudi Arabia, in a vector survey carried out by a WHO team in 1977, (Nadim et al., 1979c) in 5 areas of Saudi Arabia (Riyadh, Taif, Mecca, Bisha and Hofuf), 920 specimens of sandflies were collected from 19 localities; P.papatasi was found in all the visited areas and was the dominant species in all parts, except in Mecca and Taif where P.bergeroti was dominant. This survey did not cover all the country, omitting the southwest (Abha, Baha, Gizan).

The general taxonomy and distribution of sandflies in Saudi Arabia have been described by Lewis and Büttiker (1982). In their study, 23,491 specimens of sandflies were collected from more than 170 localities, (species of collected flies and their distribution are shown in Table 3). These consist of 10 species of Phlebotomus and 15 species of Sergentomyia. P.papatasi is reported from all the provinces.

All the investigators of the vector in the above mentioned study considered P.papatasi to be the main vector of CL in Saudi Arabia, except in Mecca where P.bergeroti was

TABLE 3 Distribution of sandflies in each province. (Adapted from Lewis and Büttiker, 1982; 1986).

Sandfly species	Distribution
P. bergeroti	all provinces
P. papatasi	all provinces
P. alexandri	western province
P. kazeruni	northwest (most common in Khayber and Alula)
P. saevus	southwest, northwest and east (rare)
P. sergenti	northwest and east province
P. major syriacus	north province
P. orientalis	mainly in mountainous area in the southern province
P. arabicus	south province
P. naqbenius	two places in Hail area and southwest
S. dreyfussi	high in south, very rare in east
S. squamipleuris	new species - very rare in east
S. adleri	south, east and central provinces
S. calcarata	south
S. christophersi	all provinces
S. clydi	all provinces
S. tiberiadis	all provinces
S. sonyae	new species in the south
S. antennata	east and west
S. fallax	all provinces
S. schwetzi	south and east provinces
S. taizi	southern province
S. africana	southern province
S. magna	new species in southern province
S. palestinensis	new species in west and northwest provinces

suspected to be the vector (Nadim et al.,1979c). Obviously this speculation depends on P.papatasi being more abundant than other species and because P.papatasi has been found to be a vector in other countries, but none of these observations alone is enough to incriminate a vector absolutely (Killick-Kendrick et al., 1981; WHO, 1984). The incrimination of P.papatasi as the vector of L.major in Saudi Arabia - Eastern province has been proved by Killick-Kendrick et al. (1985). The proof of the role of P.papatasi as the vector is provided by isoenzyme typing, fly isolates being identical to the human isolates. In addition, this species is the only species which represents the genus Phlebotomus in this part of the country (Buttiker and Lewis 1979; Killick-Kendrick et al.,1985).

The role of P.papatasi in transmission of L.major in other areas similar to Al-Ahsa is unknown and more precise documentation of vector distribution in different parts of the country is needed.

Part 2: Methods for the Diagnosis of Leishmaniasis and Identification of the Parasites

1. Introduction

The definitive diagnosis of any infection is the demonstration of parasites from the individual and in surveys. It is not always easy to identify the parasite and depends on the methods used, the density of the parasites in

the specimens and the degree of experience of the examiner. The indistinguishable morphology of Leishmania parasites makes the identification of Leishmania species more difficult. The precise identification of Leishmania parasites is a fundamental step in the assessment of the disease status in the community, treatment, determination of control measures required, and their monitoring and evaluation.

The taxonomic status of Leishmania is still not very precise. Identification of the parasite and establishment of correct taxonomy are the basic tools of any critical analysis concerning clinical, immunological, ecological or geoepidemiological surveys. The methods of Leishmania parasite identification give different levels of identification; some methods are used for genus identification and some for species identification, eg, the biochemical methods will give more details about the precise taxonomic status of Leishmania isolates and serodiagnosis is valuable for clinical and epidemiological purposes. This is very common and popular. The purpose of this section is to review the methods used for the identification of Leishmania parasites and their application in clinical diagnosis and epidemiological studies of leishmaniasis.

2. Preliminary Data for Identification

2.1 Case history

The history of the infection should include the routine

data, eg, name, sex, age, past history and history of the present condition. Geographical location of the patient is very helpful for identifying the species as each area has different species or different strains. Social background is also important as it relates to possible exposure to infective sandflies at the site of the patient's work or in the home. The size of lesion, shape and progress should be noted. If the history of the patient is ignored then the first clue for the economic and the definitive diagnosis will be missed. (I well remember talking to a physician who had missed diagnosing a case of malaria, simply because he had ignored the movement of the patient who was living in a non-malarious area but went with a religious team to a malarious area (Gizan) where he was infected. Obviously the physician ignored this trip and the patient was not diagnosed as a case of malaria simply because he came from a non-malarious area). It is essential to obtain as much history as possible from the patient and his family as often several members of one family are infected simultaneously. In the case of visceral leishmaniasis more details about the history of fever, body weight and rashes should be taken into account.

2.2 Physical examination

Careful examination of the patient is helpful to predict whether the patient has or has had leishmaniasis and in some cases this will help in identifying the initial

species until more specific methods are done. In general, Leishmania tropica causes dry lesions often leading to disfiguring scars. L.major produces moist lesions which are associated with secondary infection and are often severely inflamed and ulcerated. The physician must look carefully on examination of the patient for any lesion as some patients will not want to show any signs to the examiner, especially female patients. In visceral leishmaniasis enlargement of the spleen is the main sign to reveal the presence of visceral leishmaniasis. Other signs to be noted by the physician are enlargement of the liver and lymph glands, and chronic intermittent fever and anaemia. Obviously in areas where malaria transmission occurs the first investigation should include a search for malaria parasites in thick and thin films by a competent microscopist. I should emphasise again that the history and physical examination are the first steps towards diagnosis and identification. The following quotation seems to be appropriate here: "As a medical student, the writer (Professor Wallace Peters) used to receive regular reminders from his Professor of Dermatology that the cutaneous manifestations of syphilis were protean and that it was a bold man who would make a definitive diagnosis of the disease without laboratory evidence to confirm it" (Peters, 1985b). The same is true of leishmaniasis.

3. Morphological methods

3.1 Direct identification of amastigotes

This type of Leishmania identification depends on the demonstration of the amastigote in the lesion in a case of cutaneous leishmaniasis or from bone marrow and splenic aspirate in a case of visceral leishmaniasis. The identification by this method is at the genus level and confirms the infection of leishmaniasis. Mensural differences have been reported between different Leishmania amastigotes (Chance, 1979). The measurement of the amastigote is very tedious and does not give enough detail for each species. For this reason part of the sample taken for direct examination can be taken for the culture inoculation for further identification by biochemical and immunological techniques.

Amastigote demonstration is not easy, especially in chronic lesions (Cuba Cuba et al. 1981). A study of New World leishmaniasis showed 36% positive smears in a group of 75 patients with clinically diagnosed cutaneous forms of leishmaniasis by using direct smear (Zeledon and Ponce, 1974). In another study in Brazil, 55.1% of patients with cutaneous leishmaniasis showed evidence of amastigotes in the lesion (Cuba Cuba et al. 1981), and in Saudi Arabia, 77.8% of the total number of patients gave positive amastigotes in lesions (Abdel Fattah et al. 1982). Regarding visceral leishmaniasis, direct examination gave very good sensitivity and reproductivity. In a study in the Gizan area

in Saudi Arabia, nine patients out of ten were positive on bone marrow examination. Splenic aspiration is the diagnostic test of choice for visceral leishmaniasis in experienced hands (Ho _et al_. 1983). However, expertise both in the performance of the procedure and the interpretation of the smear is not available in every health centre. Moreover, the procedure is hazardous when performed by inexperienced personnel and where there are no facilities to observe the patient.

3.2 Culture methods

The first attempt to culture _Leishmania_ was made by Rogers (1904) when he was able to grow samples from kala-azar patients in sodium citrate solution. Nicolle (1908) cultured _L.infantum_ in tubes of Novy and McNeal's rabbit blood agar and in a simplified medium known as NNN (Novey, McNeal and Nicolle) medium. Diphasic medium of Tobie _et al_.(1950) has been successfully and widely employed for isolation and maintenance of _Leishmania_.

Modified monophasic medium and diphasic medium have been suggested for heavy and rapid growth of _Leishmania_ species by many investigators (Taylor & Barker 1968; Evans _et al_. (1984).

The principal uses of _Leishmania_ cultures are:

i. Diagnosis of _Leishmania_ infection. In some cases it is not easy to demonstrate the amastigote by direct smear, particularly in the paucity of amastigote parasite, though,

culture will help for diagnosis and confirm the infection of leishmaniasis.

ii. Leishmania cultures can be used as a source for bulk growth which helps biochemical identification of Leishmania species.

iii. Cultures have a great value in immunological and metabolic studies.

iv. In drug testing (amastigote culture).

3.2.1 Culture of promastigotes

3.2.1.1 Choice of culture media

The desirable qualities of a medium for diagnosis and isolation of Leishmania have been described by Miles (1980) as follows:

i. High sensitivity for small numbers of amastigotes or promastigotes.

ii. A liquid formulation, lyophilizable, without loss of efficacy upon reconstitution.

iii. Protection against bacterial and fungal contamination by antibiotics and fungicides.

iv. A long shelf life.

v. The capacity to be both reconstituted and seeded in the field by "Through the cap" inoculation.

vi. Low cost.

A great variety of media have been developed for the isolation and cultivation of Leishmania species. Obviously there is no universal medium in which all strains of

Leishmania will grow, and it is not necessary that a medium which is appropriate for bulk growth will be the best for initial isolation (Miles, 1980). The initial establishment of *Leishmania* spp in culture is the most critical part.

Negative culture from positive slide aspirates were obtained from different laboratories, eg, (Githure *et al*. 1984; Lightner *et al*. 1983; Abdel Wahab *et al*. 1984). The discrepant results obtained from the culture and microscopic examination can be contributed to many factors such as - (a) parasite density, (b) quality of preparation of the media and (c) the aseptic technique at the time of isolation. Before starting the culture of the parasite, we have an important step to take which will affect the outcome. This process is the isolation of the specimens from humans as well as from animals and vectors. I should emphasise again that the initial isolation in culture is the most critical step and it is almost impossible to predict which medium is going to be best suited for the growth, especially in the initial isolation (Evans, personal communication). Generally speaking, the quality of samples taken from the patient, the size of the sample and the aseptic technique are the major factors which will influence a good or bad outcome. Nevertheless, there are some media most suitable for the initial isolation, and others for bulk growth or used for transportation (Hendrickes and Wright, 1979; Rassam and Al-Mudhaffer, 1979; 1980).

3.2.1.2 Contamination of the media

Rigorous aseptic precautions are essential during culture, especially during skin culture from humans or animals. Sterility by animal inoculation (hamster) can be achieved but not for each species of _Leishmania_. Antibiotics, mainly gentamycin at 50 ug/ml of culture will provide broad-spectrum antibacterial protection to initial cultures (Evans, personal communication). Appropriate fungicides such as 5-fluorocytosine can be added where it is needed (Miles, 1980).

3.2.1.3 Temperature of incubation

More _Leishmania_ cultures are killed by heat than by cold. 22 ^{o}C is an ideal temperature for rapid growth of most _Leishmania_. The parasites will grow less readily if the temperature rises much above 24 ^{o}C (Peters, 1985b), so the culture should be protected, especially during the summer months.

3.2.1.4 Culture vessels for isolation purposes

Two types of culture vessels are most commonly used.

(a) A small screw-capped glass bottle (5 ml) (bijou bottles).

(b) Round glass tubes, either screw-capped or sealed by a rubber bung (150 ml).

Bijou bottles are easier to handle and more economic (Evans, personal communication). Disposable plastic culture

tubes can be used with some modification as it is not possible to autoclave disposable plastic tubes without destroying them.

3.2.1.5 Defined liquid medium

Chemically defined medium is normally composed of known nutrients, eg amino acids, vitamins, sugars, nucleotides and other essential growth factors. Four defined media have been developed for growing *Leishmania* promastigotes (Trager's defined medium 1957; REIII of Steiger and Steiger, 1977; Berens and Marr, 1978) and more recently MD-29 (Melo *et al*. 1985). Details of these media are given in the different publications and it is not intended to discuss these in this chapter. Cultivation of *Leishmania* in these defined media provides valuable information concerning their nutritional requirements and biochemical characteristics and immunological aspects.

3.2.1.6 Cloning

Cloning is a term for the process of growth derived from single promastigotes. This process has a great value especially for the immunologist and molecular biologist. A simple and rapid technique for cloning *Leishmania* promastigotes has been developed by Evans and Smith (1986). This technique depends on the isolation into microdrops, observation in hanging drops and cultivation in capillary tubes.

3.2.2. Amastigote cultivation

Leishmania amastigotes are the intracellular forms of the parasites which normally parasitise the macrophages. The cultivation of amastigotes is of great value when investigating processes such as macrophage-parasite interactions and drug screening.

Intracellular amastigote cultivation is more common and basically two main systems are used, (a) cultivation in peritoneally-derived macrophages and (b) cultivation in macrophage cell lines.

The amastigote culture is used mainly in research and a detailed account of the various procedures available for the cultivation of these forms can be found in an article by Chang and Fish (1983).

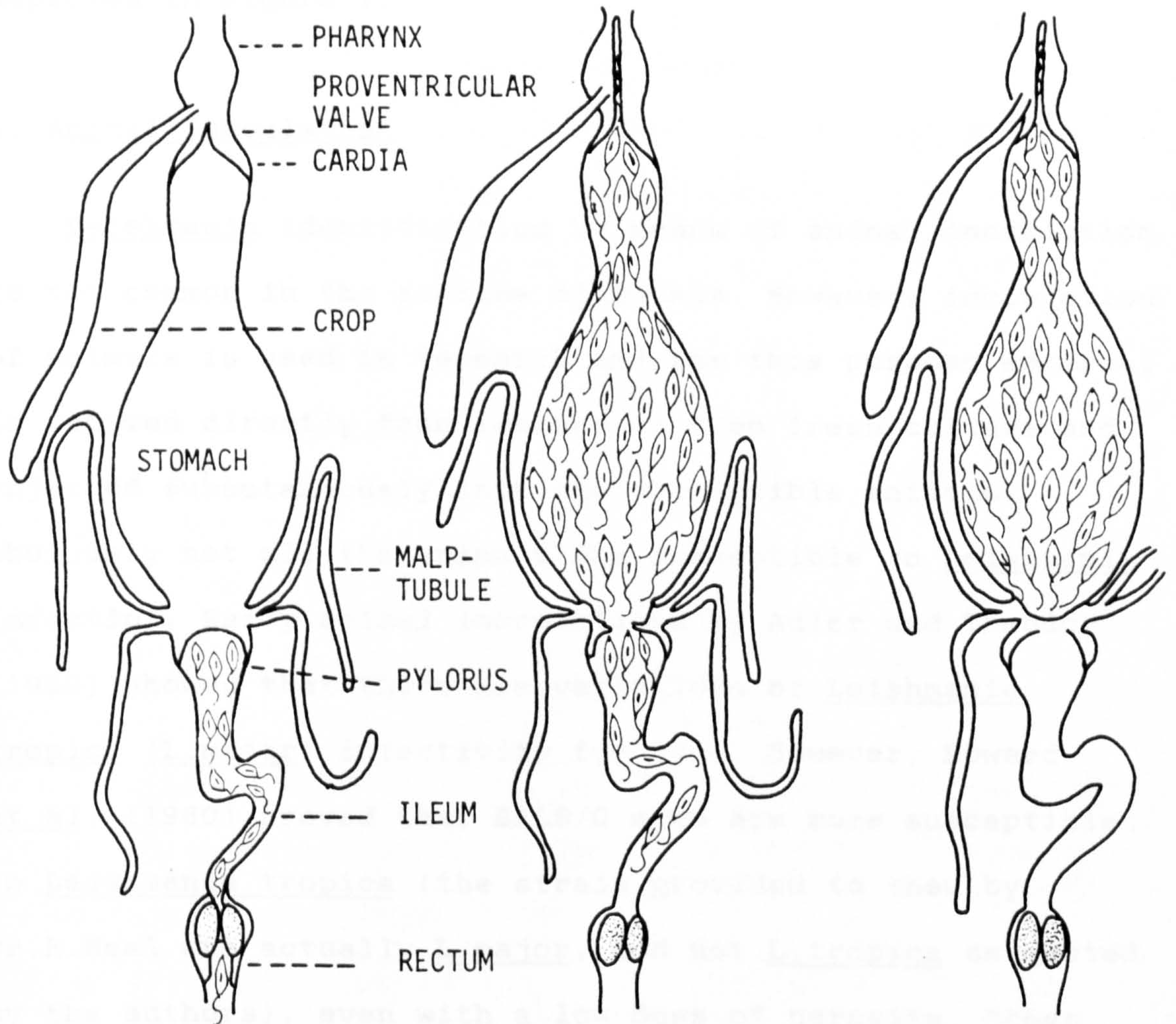
4. Site of the Development in the Vector

The pattern of development in the insect has been used as a means of categorising the parasite. Lainson and Shaw (1979) subdivided the genus *Leishmania* into three groups.

a. *Hypopylaria* Parasite is developed in the hindgut and rectum, eg reptilian *Leishmania*.

b. *Peripylaria* The parasite has a phase of reproduction in the interior part of the hindgut before migrating forward to the midgut.

c. *Suprapylaria* The parasite does not enter the hindgut, but multiplies in the midgut before



1. Hypopylaria
eg L.agami
L.ceramodactyli

2. Peripylaria
eg L.braziliensis complex

3. Suprapylaria
eg L.tropica
L.major
L.aethiopica
L.donovani
L.infantum
L.chagasi

Figure 1 A diagrammatic representation of the classification of Leishmania in the gut of sandfiles showing sites of parasite colonization.
(After Lainson & Shaw, 1987)

migrating to the pharynx, cibarium and proboscis.

The diagrammatic representation of this group is depicted in Figure 1.

5. Animal Inoculation

Leishmania identification by means of animal inoculation is not common in the routine diagnosis. However, inoculation of animals is used in research and for this purpose material is removed directly from lesions or from fresh culture and injected subcutaneously into the susceptible animals. Obviously not all the animals are susceptible to Leishmania infection. Early animal inoculations by Adler and Theodor (1930) showed that there are variations of Leishmania tropica (L.major) infectivity for mice. However, Howard et al. (1980) proved that BALB/C mice are more susceptible to Leishmania tropica (the strain provided to them by Dr.R.Neal was actually L.major, and not L.tropica as quoted by the authors), even with a low dose of parasite. Other strains of mice such as CBA mice are resistant to even high doses of promastigotes. Leishmania donovani complex may be inoculated in hamsters and the infection will develop in liver and lymph glands within 3 to 4 weeks; but not all strains are infective to hamsters (Peters, 1985a; Bradley and Kirkley, 1977). Also hamsters can be used as a model for the isolation of Leishmania major (Mahmoud et al. 1985), and for the Leishmania braziliensis group (Dr.R.Neal, personal

communication).

It is clear that the inoculation of animals is not commonly used for diagnosis but mainly for drug testing, biochemical, immunity and vaccination studies. The genetic variation between experimental animals as used for *Leishmania* parasites should be taken into account for any design of animal experiments (Bradley, 1987). Footpad inoculation may prove to be much quicker than cutaneous inoculation.

6. Biochemical Methods

6.1 Introduction

In the past the criteria for taxonomy of *Leishmania* parasites and the diagnosis of the disease were based on extrinsic characters of *Leishmania* parasites (morphological, clinical and epidemiological). Unfortunately these methods could not separate the *Leishmania* from the species and subspecies (Chance 1979; Peters 1985b). It is extremely difficult, if not impossible to distinguish the different species of *Leishmania* on morphological criteria (Peters *et al*. 1983). Clinical and geographical characters may overlap and epidemiologically this can be confusing (Evans *et al*. 1984).

The advent of biochemical techniques to explore the *Leishmania* taxonomy, and more details about extrinsic characters give a clearcut separation between the different species of *Leishmania* parasites. There are a wide range of biochemical methods that have been used for identification

of Leishmania, some of them are adapted as routine methods eg, isoenzyme techniques, and some are under investigation to be adapted for routine work eg, DNA probe and monoclonal antibodies.

The isoenzyme electrophoresis is the more popular and most widely applied, being less expensive and more economic (Peters *et al.*,1983 and Le Blancq *et al*.1986). Until now most of the Leishmania isolates are tested against up to 12 enzymes (Le Blancq and Peters 1986a,b, and Evans 1984) and to some extent less than this number (Aljaboori 1980 and Al Taqi *et al*. 1978).

6.2 Isoenzyme techniques

6.2.1 The principles of isoenzyme electrophoresis

The migration of molecules or charged particles under the influence of an electric field is the main principle theory of electrophoresis. The isoenzyme is a molecular form of enzymically active protein, each protein has an isoelectric point (IEP) where the molecular has positive and charge balance (neutrality point) depending on the required condition. The protein in buffer solution at pH above or down its IEP maintains a negative or positive charge respectively. Each isoenzyme has his own specific buffer content and specific pH which keep the enzyme stable.

Based on the above principles of electrophoresis, the soluble extract of the samples obtained can be applied as a narrow zone to the strip of inert supporting matrix (eg

cellulose acetate, gels of starch) and forming a bridge between the two electrodes from a current to pass through it. The mobility and separation of isoenzyme bands depends on the voltage used, temperature and buffer contents, buffer pH, porosity of the supporting matrix.

6.2.2 Visualization mechanism of isoenzyme bands

Several methods have been devised for the detection of isoenzymes separated by electrophoresis, among these are:

6.2.2.1 Dehydrogenase reaction

In dehydrogenase reaction, some are straightforward dehydrogenase enzymes, eg malic enzyme, whilst others are not, eg phosphoglucomutase and require the use of linking dehydrogenase enzymes and in some cases, several additional linking enzymes as in the case for enolase enzyme.

The visualisation of these isoenzyme patterns depends on detecting the formation of either the oxidised or the reduced form of the coenzyme as shown in the reaction.

NAD(P)	NAD(P)H+
oxidised	reduced
non-fluorescent	fluorescent

The coenzyme fluorescent under U/V light in the reduced form only. In the reaction right to left the NAD(P) formed is detected as a dark band against fluorescent backgound of unreacted (NAD(P)H.

6.2.2.2 Hydrolysis

In this method, 4-methyl umbiliferone derivatives are

used as substrates to detect certain hydrolysis of substrates and produce a brilliant fluorescent band under UV light, easily visualised. The application of developers to plates are done by -

i. Applying it in the form of soaked filter paper
ii. Pouring it on the plate as molten agar
iii. Flooding the plate with developer solution

6.2.3 Isoenzyme methods

The isoenzyme technique was first introduced as a method for characterising serum proteins. In the early 1970s most of these methods were adapted for Leishmania isoenzyme characterisation (Gardener and Howells 1972, Gardner et al. 1974 and Kilgour et al. 1974). In general the isoenzyme electrophoresis can be run by different methods based on the previous principle eg paper electrophoresis, polyacrylamide gel, starch gel and cellulose acetate. The most popular methods in Leishmania isoenzyme characterisation are cellulose acetate and thin layer starch gel. Both methods are tested in large scale studies (Lanham 1982; Le Blancq and Peters 1986a,b,c; Peters et al.,1985). The cellulose acetate method is more easy to carry out in the field (Lanham 1982), while the thin layer starch gel method seems to be more efficient for isoenzyme resolution (Le Blancq and Peters 1986b; Godfrey 1985 and Miles 1985). Each method has its own advantages and disadvantages. Table 4 shows the advantages and disadvantages of both methods. The number of enzymes which are mostly used for examination of any

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TABLE 4 Advantages and disadvantages of TSE and CAE methods

	Thin layer starch gel electrophoresis (TSE)	Cellulose acetate electrophoresis (CAE)
The matrix	Starch which is not a defined medium	Cellulose acetate which is a defined medium
Preparation of matrix	Starch gel must be used within 24 hours of preparation	It can be prepared as a stock for use when needed and it is commercially available
Tanks	Heavy and cumbersome and very hard to carry in the field	Light and easy to carry in the field
Cooling system	Needs special apparatus such as a cooling pump and a refrigerator with cooling plate	No cooling equipment, water or ice between the tank compartment is enough
Energy source	Good electricity source	Electricity source or portable electric motor
Sample application	The plate is loaded with sterilised thread which is impregnated with the samples. Therefore it requires larger samples and more time	With direct applicator which is much easier, quicker and requires small amounts of material
Running time	It takes between 1 - 4 hours depending on the enzyme and the voltage used	Between 20 - 45 minutes depending on the enzyme and voltage used
Enzyme resolution	Highly efficient	Less efficient

isolates are: malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (6PGD), superoxide dismutase (SOD), asparate aminotransferase (ASAT), alanine aminotransferase (ALAT), pyruvate kinase (PK), phosphoglucomutase (PGM), esterase (ES), nucloside hydrolase (NH), proline imino pepto-dipeptidase (PEPD), mannose phosphate isomerase (MPI), and glucose phosphate isomerase (GPI). This could give clear separation between each species in both Old and New World leishmaniasis (Evans et al.,1984; Le Blancq et al.,1986). The term enzyme profile or zymodeme defined as "the population of parasites which are different in one or more of enzyme mobility from the original reference stock of the species or subspecies. Comparison between the patterns of enzyme profile (zymodeme) are simple by direct comparison. The profiles of isolates may not match exactly that of the marker but there are no difficulties in assigning the isolate to the appropriate taxon (Le Blancq et al.1986). Each species contains different zymodemes (London zymodeme) eg, L.major has 6 zymodemes. Different isoenzyme mobilities imply genetic differences between parasite population (Gibson and Miles (1985). The distinction of L. mexicana from L.braziliensis by means of 10 out of 14 enzymes has confirmed the major differences between them (Miles 1985). Meanwhile the L.braziliensis braziliensis has been separated from the other two members of the braziliensis group (L.b.guyanensis, L.b.panamensis) by 4 out of 10 enzymes (Evans et al.1984). In the Old World, L.major and

L.tropica are identical in only one out of 13 enzymes (Le Blancq et al.1986). L.aethiopica is distinct from other cutaneous leishmaniases in the Old World by 11 enzymes out of 13 (Le Blancq et al.1987).

Regarding the visceral leishmaniasis in the Old World and New World the causative agent, at present not separated to distinct species and still considered to be a member of the L.donovani complex (Schnur et al. 1981).

However, Lainson and Shaw (1987) treat the New World visceral leishmaniasis as separate species under the name Leishmania chagasi (Cunha and Chagas, 1937), separated from L.donovani and L.infantum in 5 isoenzymes.

In Old World leishmaniasis, Le Blancq et al.(1986a) demonstrated marked heterogeneity in the parasites of L. donovani - L.infantum complex.

Implementation of isoenzyme techniques in epidemiological studies give clear, understanding about the geographical distribution of Leishmania species and incrimination of both vector and reservoir of the disease in different parts of the endemic area. In Saudi Arabia more than 75 isolates have been identified by using up to 13 enzymes, revealing the presence of L.major as the dominant species in the Eastern province of the country with Ph. papatasi as vector and Psammomus obesus as the main reservoir (Peters et al.1985; Killick-Kendrick et al.1985; El-Bihari et al. 1987). In Iraq using up to 7 enzymes (Aljeboori and Evans, 1978) a very useful conclusion could

be drawn about the epidemiology of *Leishmania*, when they found that *L.major* and *L.tropica* do exist in Iraq, where the past belief was that *L.tropica* is the only species to exist. In Kuwait, Al-Taqi and Evans (1978) characterised a human isolate by means of isoenzyme technique and that the *L.major* is the dominant species followed by *L.tropica*.

In the New World, the etiological agent of leishmaniasis has been obtained about their epidemiological pattern (Arias *et al*. 1985; Cuba Cuba *et al*. 1985), and three distinct agents, *L.m.amazonensis*, *L.b.braziliensis* and *L.b.guyanensis* are considered to be responsible for cutaneous leishmaniasis in the Amazon Basin (Miles 1985; Evans 1984).

6.3 Other biochemical methods

6.3.1 Monoclonal antibody technique

Identification of antigenic molecule and analysis of immunological relationships between different species of *Leishmania* parasite have benefited enormously from the development and use of monoclonal antibodies.

Monoclonal antibodies for *L.braziliensis* complex have been reported by McMahon-Pratt and David (1982), Jaffe and McMahon-Pratt (1983) reported four monoclonal antibodies (T-1 through T-4), which were produced to membrane-enriched preparation of *L.major* promastigotes, one of these monoclonal antibodies (T-3) react with both the amastigotes and promastigotes of the parasite, the others were found to be for promastigote stage (De Ibarra *et al*. 1982) reported

monoclonal antibodies specific to L.major and bound to cell surface of promastigotes and to parasitized macrophages.

It is clear that by using the species-specific monoclonal antibodies we will have an efficient rapid diagnostic tool which may replace some of the current methods which are both cumbersome and time consuming.

6.3.2 DNA analysis

Implementation of DNA analysis as a diagnostic and taxonomic method for leishmaniasis has been discussed by Barker et al. (1982), Chance (1979) and Newton (1976).

The buoyant density of DNA (nDNA and kDNA) was of limited use due to the lack of sensitivity to Leishmania species and the costly apparatus.

DNA hybridization (DNA probe) is the most promising method, in practise the most used is the kinetoplast DNA (kDNA). In discussing the future of DNA probe, Barker (1987) stated that: the number of recombinant DNA probes capable of distinguishing between species and complexes of Leishmania may now be in the region of 15000. Preparation of such probes is now relatively simple and cheap, almost a routine exercise. With them, the identification of ambiguous slides - taken from culture, a clinical lesion or sandfly gut - is now possible. Use of such probes will have two main advantages. They will aid clinical diagnosis, opening the way for earlier specific treatment and they will greatly assist epidemiologists.

In conclusion, the isoenzyme technique and other

biochemical methods proved of great value for identification of leishmaniasis, epidemiological characerisation, follow-up of the chemotherapy and better standing for proper control measures.

7. Immunological Methods

7.1 Serodiagnosis

A variety of immunodiagnostic methods have been used for different tropical infections including leishmaniasis and the large number of methods suggest that none are universally applicable and each of the methods have certain advantages eg indirect immunofluorescence is sensitive but only group specific; complement fixation test is easily performed but insufficiently specific (Hommel *et al*. 1978). Criteria for diagnostic methods, particularly from one to be employed in developing countries are as follows (Draper and McLaren 1981).

1. High level of sensitivity and specificity
2. Ease of simple collection
3. Socially acceptable
4. Cost effective
5. Ease of test operation
6. To be quantitative
7. Suitable for use in field laboratories

IFAT and ELISA are the more promising tests which meet some of these criteria. The IFAT has been well evaluated and found to be of particular use for the diagnosis of

visceral leishmaniasis and other protozoal infection, both in individual clinical diagnosis and epidemiology. In cutaneous leishmaniasis especially in the Old World, the antibody level is low and commonly difficult to demonstrate by IFAT (Edrissian _et al_.1981), in cutaneous leishmaniasis particularly _L.major_. The _Leishmania_ antibody level decreases after recovery (Endrissian _et al_. 1981) The time interval between the healing of the cutaneous lesions and the serological examination has been found significantly shorter in the group with high FAT titres than in the group with low or no titres (Menzel and Bienzle 1978). Negative results in sera from patients with parasitologically confirmed cutaneous leishmaniasis have been reported (Hommel _et al_. 1978). Serological tests are therefore not employed routinely in the diagnosis of cutaneous leishmaniasis.

Visceral leishmaniasis is characterised by the massive production of immunoglobulin, mainly IgG. The presence of specific antileishmanial antibodies can be demonstrated by a variety of serological methods such as formol gel test and complement fixation test. ELISA and IFAT are the most sensitive techniques (Hommel _et al_. 1978). In India, Srivastava _et al_. (1984) compared the specificity and sensitivity of ELISA and IFAT for detection of antibodies of kala-azar and they observed that ELISA was more sensitive in detecting antibodies in the early stage than IFAT.

The potential value of ELISA in the clinical diagnosis and epidemiological study of visceral leishmaniasis has been

evaluated by many investigators (Hommel _et al_. 1978; Pappas _et al_., 1984; Prasad _et al_. 1980; Srivastiva _et al_. 1984) and the result shows that the ELISA is highly sensitive-specific, accurate and economic for the diagnosis of visceral leishmaniasis. A micro enzyme linked immunosorbent assay utilising antigen dotted on to nitrocellulose filter discs (Dot ELISA) was developed for rapid diagnosis of visceral leishmaniasis by Pappas _et al_. (1983a & b). The advantages of this method are - inexpensive, visually read (blue dot on white background) and very small amount (1 ul) of antigen is used. This method (dot ELISA) has been analysed by the same authors (Pappas _et al_. 1984) and they conclude that this method is stable, sensitive for visceral leishmaniasis in clinical diagnosis and the epidemiological field. In general the serodiagnosis has a great value in epidemiological studies. Its value for diagnosis will remain questionable until we have a highly pure antigen. Nevertheless its use in clinical diagnosis is essential for confirmation of diagnosis. It is hoped that the production of monoclonal antibodies will help to improve the immunodiagnostic method and the taxonomy of the organism.

7.1.1 Antigen purification in serodiagnosis

The antigenicity complex of the _Leishmania_ parasite and other parasitic organisms hamper the application of sero-diagnosis in terms of specificity, sensitivity and cross-reaction. Nevertheless, antigenic structures of _Leishmania_

have received considerable attention to improve immunodiagnosis and vaccination studies. Soluble antigen extracted from cultured promastigotes is the main source of _Leishmania_ antigen and generally named excreted factor (EF) The suitability of different subcellular antigens from a serodiagnostic standpoint has been shown that the fraction F5 was found to be most suitable for all the immunodiagnostic tests (Mukerji _et al_. 1984). Intact promastigote in formalin solution has been employed by Elamin _et al_. 1985) and they demonstrated that the use of intact promastigote as an antigen can be as good as or better than the soluble antigen and increase the specificity of ELISA.

The advantages of intact antigen as described by Elamin are (1) promastigote antigen requires no processing beyond washing and can be stored for about 45 days at -20°C or -4°C, (2) using intact promastigotes as antigen is that antibodies to surface antigens, perhaps with interesting functions, will be more likely to be detected. However, the intact antigen, shows cross reaction and more evaluation of this antigen is needed. In IFAT whole parasite antigens are usually employed which give good reproducibility but effect the specificity.

7.1.2 Collection of samples for serodiagnosis

The majority of tests are done on plasma or serum in clinical diagnosis in the hospitals; there is usually no problem in obtaining this from an intravenous blood sample. Therefore

a number of methods have been devised by which a finger prick can be used to collect blood samples into 50 or 100 ul capillary tube either with or without anticoagulant coating, which is then centrifuged and the part containing the plasma broken off, sealed and stored in the cold (Draper and Lillywhite, 1985). The collection of large spots of capillary blood onto absorbent paper (Whatman filter paper No.4) from which a disc is later cut and eluted has been recommended as a tool for seroepidemiological studies.

The blood spot technique has the advantage that the samples occupy little space, can be easily transported and maintain their antibodies for years. The limitation of the blood spot method has been reported by Werner and Frosner (1985). The limitation is that the low titre antibodies or antigen in serum will not be detected.

Diagnostic tests are generally judged on the basis of their sensitivity and specificity. Such evaluations are essential. The predictive value of a test which depends upon the disease prevalence as well as the sensitivity and specificity is the most important measure for determining the usefulness of a test under field conditions.

7.2 Leishmanin skin test (Montenegro test)

7.2.1 Cutaneous leishmaniasis

Cell mediated immune response in cutaneous leishmaniasis can be demonstrated by the use of an intradermal skin test using fractionated cultured promastigotes as antigen. Test

is specific for infection within the genus _Leishmania_, but does not differentiate between different species of _Leishmania_ (Peters, 1985b). The leishmanin test is useful as a diagnostic test in non-endemic areas, and not so useful in endemic areas where the positivity rate is high (Manson-Bahr, 1984). The leishmanin test is especially valuable as an epidemiological tool (Morsy and Hawary, 1974). The test is simple and socially acceptable; using a tuberculin syringe, 0.1 ml of antigen is injected intradermally on the forearm. A control injection is given with separate syringe about 10 cm below. The site is observed after 24 and 48 hours, usually about 72 hours. A positive reaction is indicated by the presence of a classical wheal and flare reaction at the site of antigen. It appears first about 24 hours and is maximum at 48 hours (size usually about 5 mm diameter).

A positive reaction is indicative of a past or current infection. A negative response may be found in patients with early lesions usually less than one month (Peters 1985b). False reactions may occur, but are unlikely to be seen in patients in the Middle East (Peters 1985b). A survey has been conducted between pupils in primary schools in the Eastern Province, [Saudi Arabia National Leishmaniasis Research Program (NLRP) 1985, unpublished report]. A total of 272 pupils were given the Leishmanin test, the findings of this test showed surprisingly high positivity rates (69% - 90%), (Figure 2) among those who had no active ulcers or

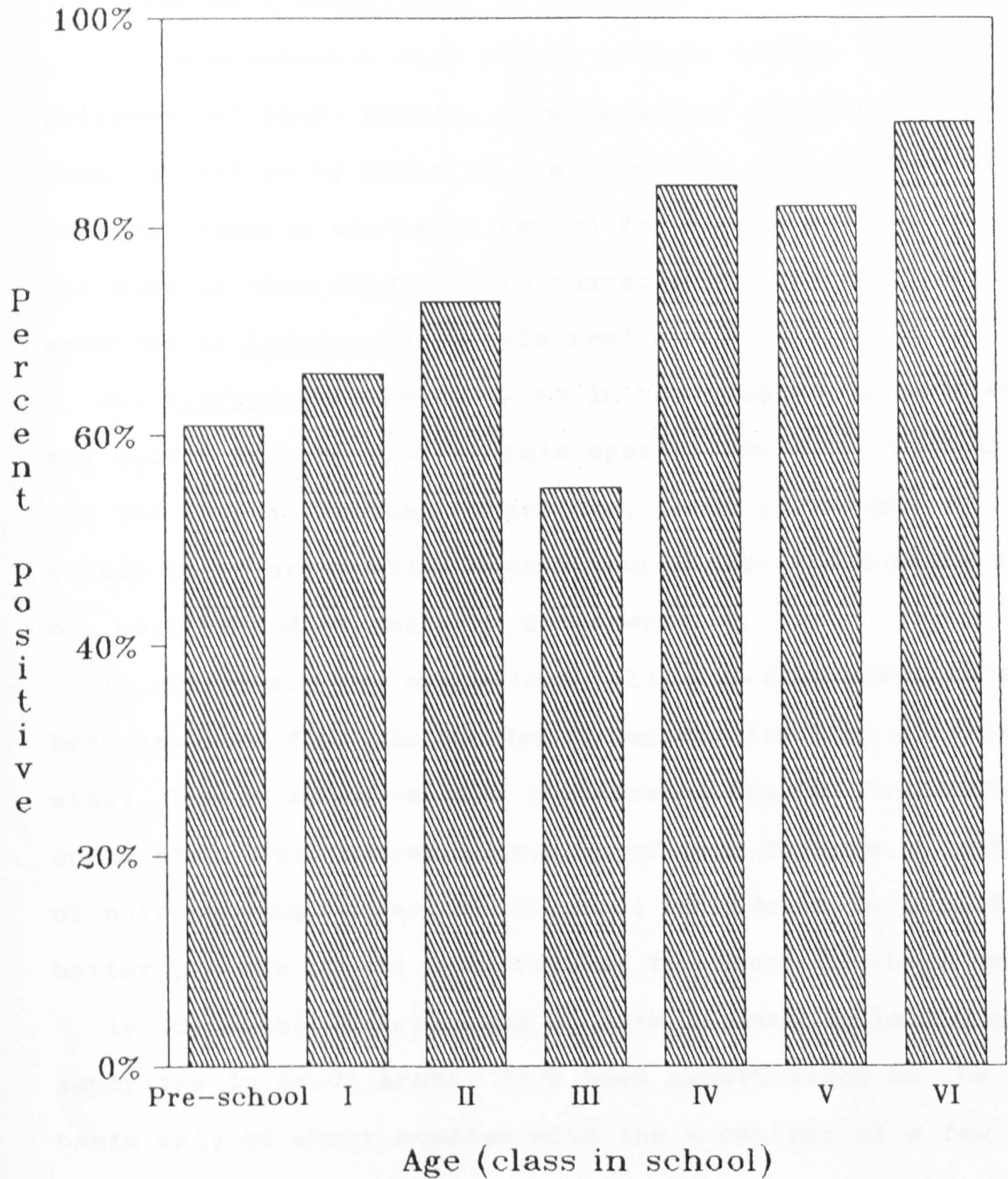
definite scars. This result explained by the investigators that (a) children are probably exposed to the disease early in life and this could have given them some immunity against later exposure and (b) these infants may sometimes receive an earlier exposure to a possibly different strain of *Leishmania* which is not pathogenic and gives a good protection (most probably infected with *L. arabica*, Peters *et al*. 1986).

7.2.2 *Visceral leishmaniasis*

In the Old World the Leishmanin test is negative during acute and untreated cases, but becomes and remains positive following resolution of clinical symptoms (Peters 1985b). The test becomes increasingly positive with age and duration of residence in an endemic area and is more positive in males (Smith 1984). A study in Kenya showed (Ho *et al*.,1982) that the age related increase in skin reactivity was not significant. In American visceral leishmaniasis, a recent study (Reed 1986) indicated that the leishmanin test using an extract of *L.donovani chagasi* is a sensitive and specific indicator of previous infection with American visceral leishmaniasis.

Fig. 2. Leishmanin positivity rates in children in Al-Jisha, Eastern province

(Source: Annual report of the NLRP team, Epidemiology unit, 1985)



CHAPTER II SITE, MATERIALS AND METHODS

Part 1: General Study Plan

1. Research Objectives

These were essentially as follows:-

i. Leishmaniasis is a highly endemic disease in the southwest of Saudi Arabia. The causative organism has not been identified by means of the isoenzyme technique which is the most readily available method for identification. One of the aims of this study was to characterize the aetiological agent(s) of *Leishmania* in this region.

ii. *L.tropica* seems to exist in the mountainous area but the vector and the host of this species are still unclear in all the endemic regions. Therefore, their investigation should be of great value both to control of the disease in our country and to medicine in general.

iii. With very few exceptions, all kala-azar cases have been reported from the southwest region, the area of this study. The parasite causing the disease has not been well documented. Full characterization of this disease by means of both serological and biochemical methods would give a better picture of the structure of the focus of the disease.

iv. Seasonal distribution of both leishmaniasis and the sandflies in Saudi Arabia have been hypothesised on the basis only of short studies with the exception of a few

regular monthly studies that have been made in the Eastern province of the country. This research was, therefore, designed to cover this topic by regular epidemiological and entomological follow-ups.

v. Comparative studies of the sandfly populations in two different ecological areas were to be carried out to examine the effect of geographic features on the seasonal abundance of the sandflies.

vi. Overall, it was hoped that this research would give practical guidance to the control of the disease.

2. Time and Period of Study

The writer joined the Department of Medical Protozoology at the London School of Hygiene and Tropical Medicine on 5th November, 1985 where he started his practical training on the different aspects of *Leishmania*.

The field studies continued for one year, commencing in October 1986 and finishing in October 1987. All the objectives mentioned above were met due to the proper training, personal experience, help and advice of my supervisor.

3. Preparation Phase for the Field Study

The preparation for the field study began during my first year's training at the London School of Hygiene and Tropical Medicine. My supervisor, while he was participating in a scientific meeting in Riyadh, Saudi Arabia, travelled

to Abha city, the capital of the Asir region, in order to see the available facilities in the study area. He met the staff of the Ministry of Health and some of the staff of the Abha Medical College, a branch of the King Saud University at Riyadh and they all agreed on the use of their available facilities for the research.

This study was financed by the Ministry of Health, who authorized the field work, and informed The Regional Environmental Department and other Ministry departments involved in the field work. With the agreement of the Ministry of Health, the staff of the *Leishmania* departments in the southern region were asked to cooperate, and to allow the use of the laboratory in the Asir *Leishmania* department as a central area for collections made during the field study. A reliable car was provided by the Ministry of Health for the field activities.

Valuable field training was received by the writer during two weeks in Al-Ahsa Province, particularly relating to the entomological and reservoir studies, from members of the SANCST/King Faisal University, National Leishmaniasis Research Programme (NLRP) teams. In addition to the field experience, I was able to borrow materials and equipment from them.

Personal contact and field visits to the major health centers, mainly the *Leishmania* centers and hospitals in the study area were made for the following purposes:-

(a) To gain experience about the *Leishmania* cases and

their distribution.

(b) To find out what facilities exist in each province of the study area.

The reasons for Abha City being chosen as the centre for this study were as follows:

(i) Abha is the main city in the southwest and is the center of the study area. It is 300 km from Al-Baha and approximately the same distance to Gizan.

(ii) As mentioned previously, this study was to cover both visceral leishmaniasis (VL) and cutaneous leishmaniasis(CL). The prevalence of the two diseases in two different aetiological areas in the Asir province would meet most of our objectives. Other provinces either have more CL with few cases of VL (Al-Baha province) and/or have more VL than CL as in the Gizan province.

(iii) Abha has a medical college as a branch of King Saud University, Riyadh, with available facilities for the study.

4. Establishment of the Abha Laboratory

The laboratory facilities at the Abha *Leishmania* department were set up by the writer as follows:-

There were originally two sections in the Abha *Leishmania* department, one for data collection from the Public Health Centers and hospitals, the other being is the medical section for patient diagnosis and treatment.

The laboratory facilities were available for simple direct smear examination only. Culture technique was not

available. There were no entomological studies except random collections by sticky traps to be sent to the Leishmania Center in Riyadh for classification.

The first step in the study was to set up the laboratory and train the staff as there was only one skilled laboratory technician working on *Leishmania*, the others being unskilled and untrained for this type of work.

The local health authority in Asir Province and the Preventive Medicine Department in the Ministry of Health Riyadh decided to provide two more rooms to the *Leishmania* department for use as an entomology laboratory. Also one entomological technician and one health inspector were transferred to the *Leishmania* department. Training for the *Leishmania* group in Abha was carried out by the writer.

The laboratories were equipped with incubators, refrigerators, autoclaves, microscopes (ordinary binocular and dissecting). The equipment and instruments were borrowed from the *Leishmania* Center in Riyadh, from King Faisal University, Al-Ahsa and from the College of Medicine, King Saudi University, Abha.

At the beginning of the study a visit was arranged for my supervisor, Professor Peters, to check all the facilities mentioned above for the field work in the study area.

The preparatory steps for fieldwork and the establishment of the laboratory were undertaken in 45 days from the middle of October 1986 to the end of November 1986. The unlimited support of the Ministry of Health was a great help in enabling me to set up this laboratory as planned.

Part 2: Landscapes and Populations of the Study Area.

1. Location

The southwest region of Saudi Arabia includes the Provinces of Asir, Baha and Gizan, approximately between the latitudes of 22° 44'N and 15° 15'S and between the longitudes 40° 45'W and 44° 00'E.

Other Provinces such as Mecca and Najran have some districts extending to the borders of the study area (see map 1 showing the administrative setting of the study area).

2. Topography

The southwest of Saudi Arabia can be divided into four topographic zones from west to east (map 2). These are:

i. The Red Sea coastal plain

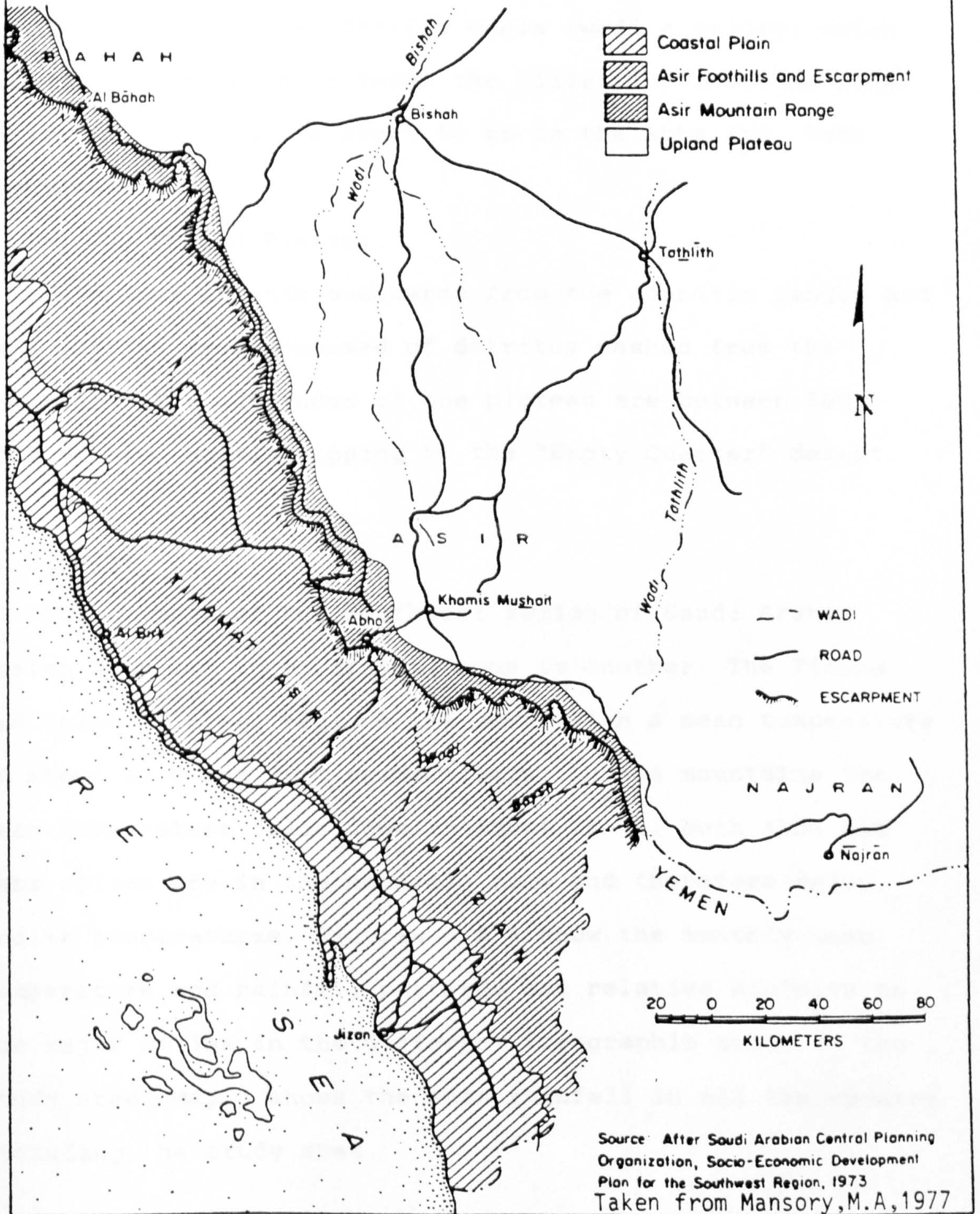
ii. The Asir escarpment foothill

These two zones are known together as Tihama. It reaches its maximum width of forty kilometres in Gizan Province. East of the true coastal plain we find the area of the Tihama hills which are composed of many parallel ridges with heights starting at 100 to 200 metres in the west and rising eastward to elevations around 1600 meters near the escarpment.

iii. The mountain ranges

These are known as the Asir or Al-Hijas mountains. Locally these mountains are known as Alsarat (which means the high mountains). They extend from Tayif in the north to

Map 1
Aministrative setting of the study area


Map 2
PHYSIOGRAPHIC DIVISIONS
of the Southwest Region of Saudi Arabia
Coastal Plain
Asir Foothills and Escarpment
Asir Mountain Range
Upland Plateau
B A H A H
Al Bāhah
Bishah
Wadi Bishah
Tathlith
Wadi Tathlith
A S I R
Khamis Mushait
Abha
Al Birk
TIHAMAT ASR
WADI
ROAD
ESCARPMENT
N A J R A N
Najrān
YEMEN
RED SEA
Jizan
20 0 20 40 60 80
KILOMETERS
Source: After Saudi Arabian Central Planning Organization, Socio-Economic Development Plan for the Southwest Region, 1973
Taken from Mansory, M.A, 1977

the Yemen border in the south. The western slopes of the mountains fall away precipitously from about 3,000 metres above sea level around Al Saundah, near Abha towards the Tihama hills. The eastern slopes are more gentle and divide into a series of green fertile wadis (wadi = valley) which allows for settlement between the hills. The mountain zone varies in width but is about 50 km in the Abha and Baha Provinces.

iv. The Upland Plateau

This zone extends eastwards from the mountain ranges and is a flatter area composed of detritus washed from the mountains. The altitudes of the plateau are between 800 - 1500 metres, gently dipping to the "Empty Quarter" desert.

3. Climate

The climate of the southwest region of Saudi Arabia varies from one topographical zone to another. The Tihama and Upland Plateau regions are warm, with a mean temperature of about 31 ^{o}C and 26 ^{o}C respectively. In the mountains the mean temperature falls down to about 16 ^{o}C. Both Abha and Baha cities are in the mountain zone and therefore enjoy cooler temperatures. Figures 3 & 4 show the monthly mean temperature and rainfall patterns and relative humidity to the major cities in the different topographic zones in the study area. Map 3 shows the main rainfall in all the country including the study area.

Fig. 3. Mean monthly temperature (Celsius) and rainfall (mm) at stations in mountainous and lowland parts of the study area

Temperature (lines) Rainfall (histograms)

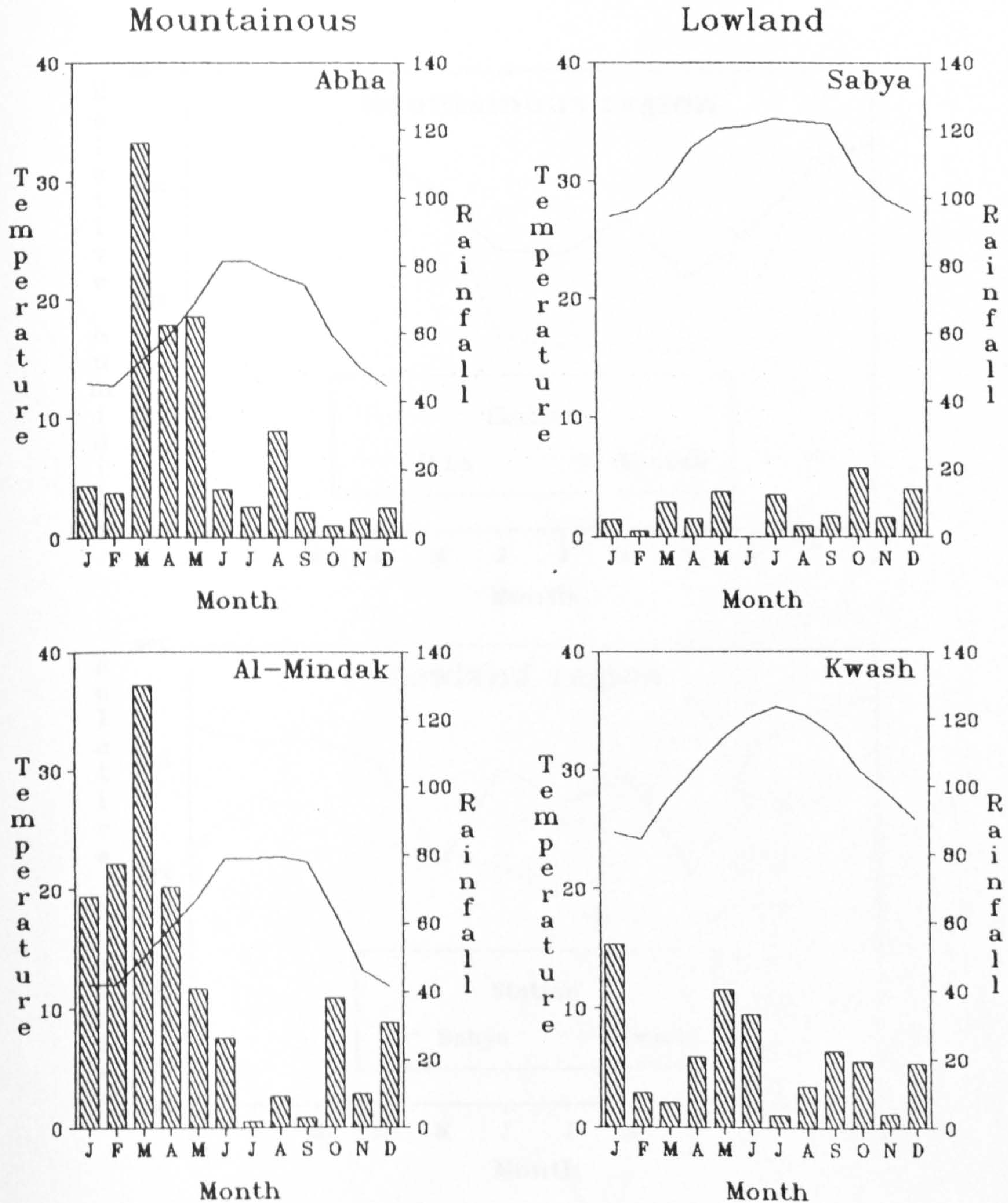
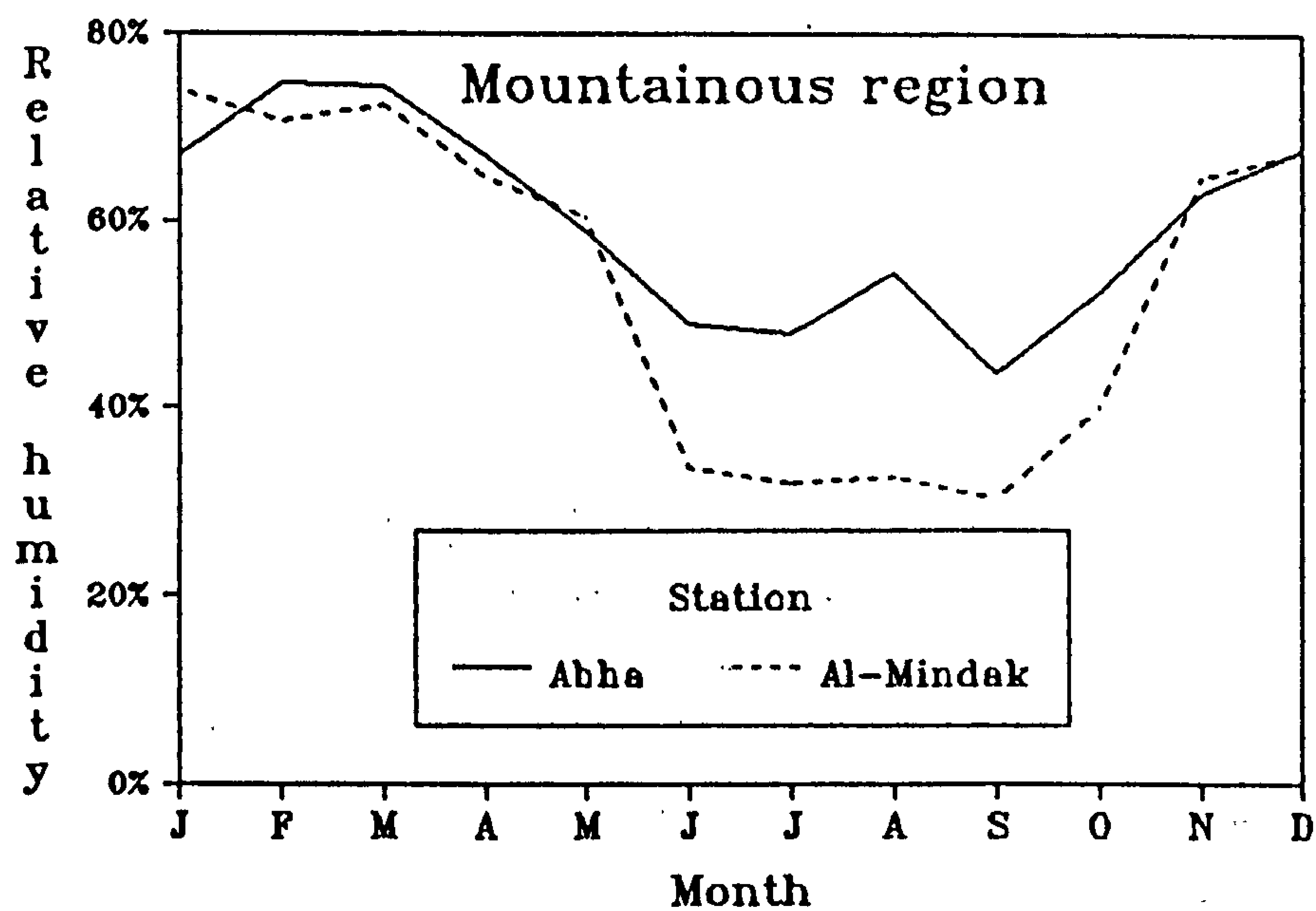
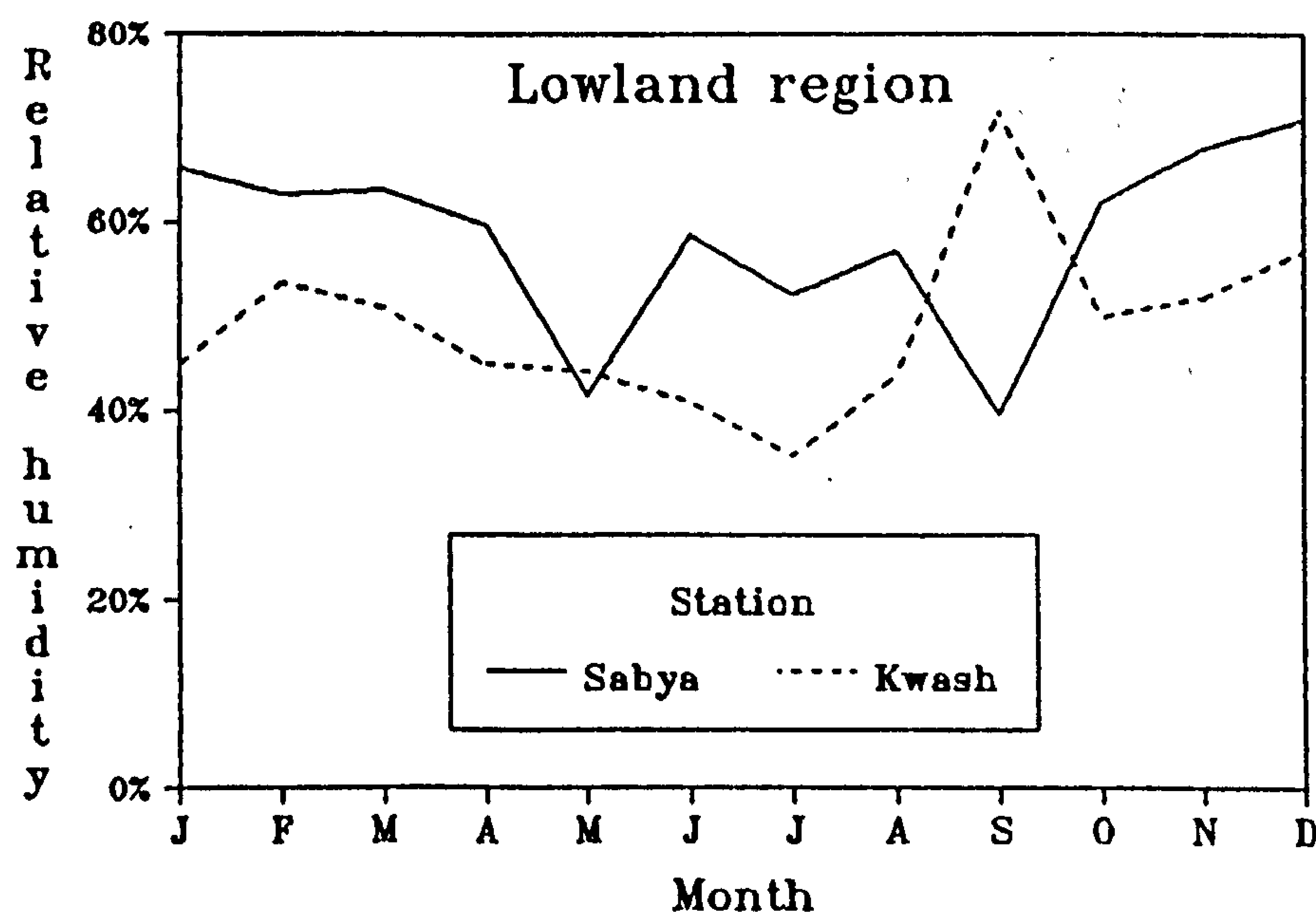
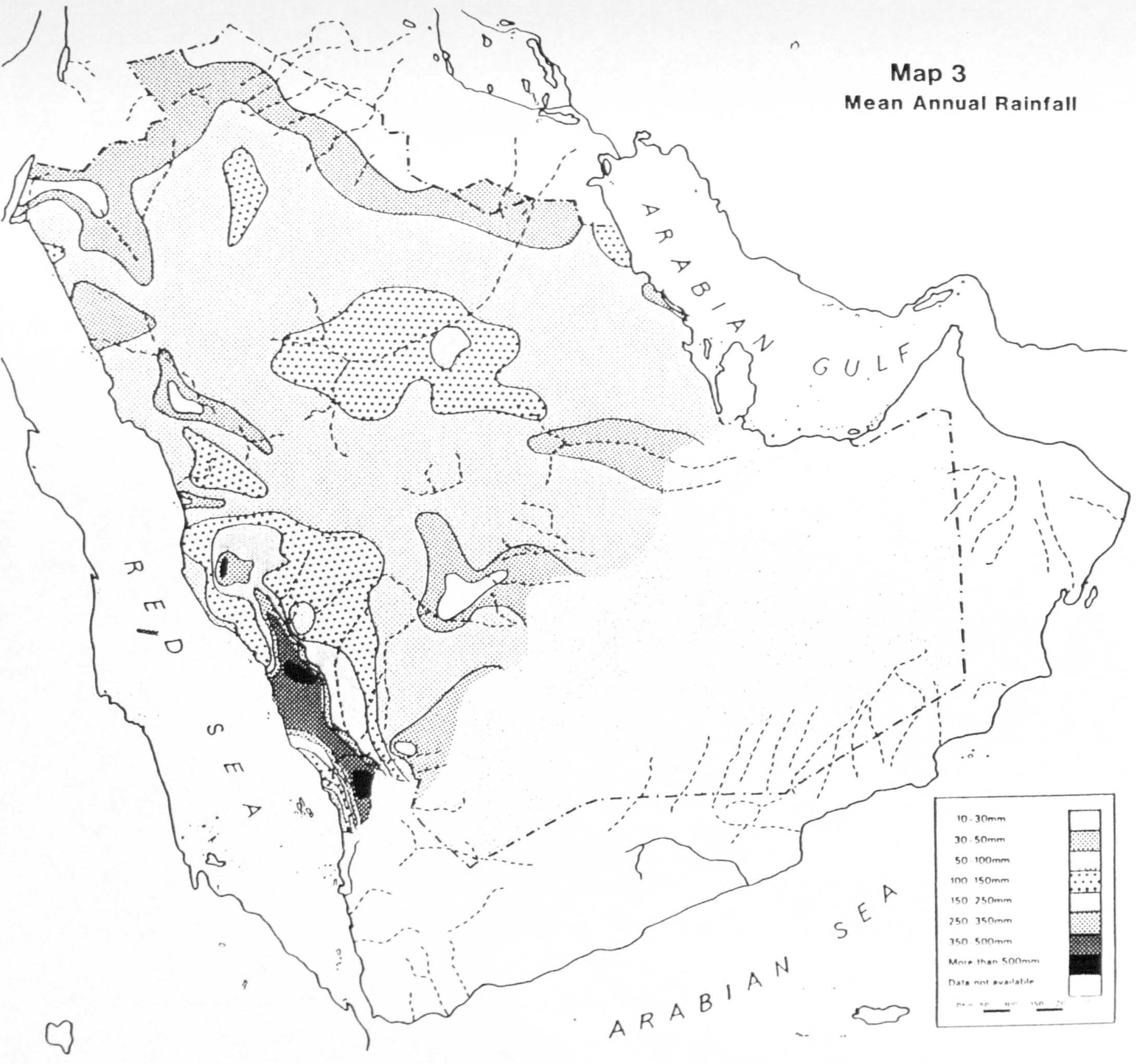


Fig. 4. Mean monthly relative humidity at stations in mountainous and lowland parts of the study area






Map 3
Mean Annual Rainfall
ARABIAN GULF
RED SEA
ARABIAN SEA
10-30mm
30-50mm
50-100mm
100-150mm
150-250mm
250-350mm
350-500mm
More than 500mm
Data not available

4. Soil

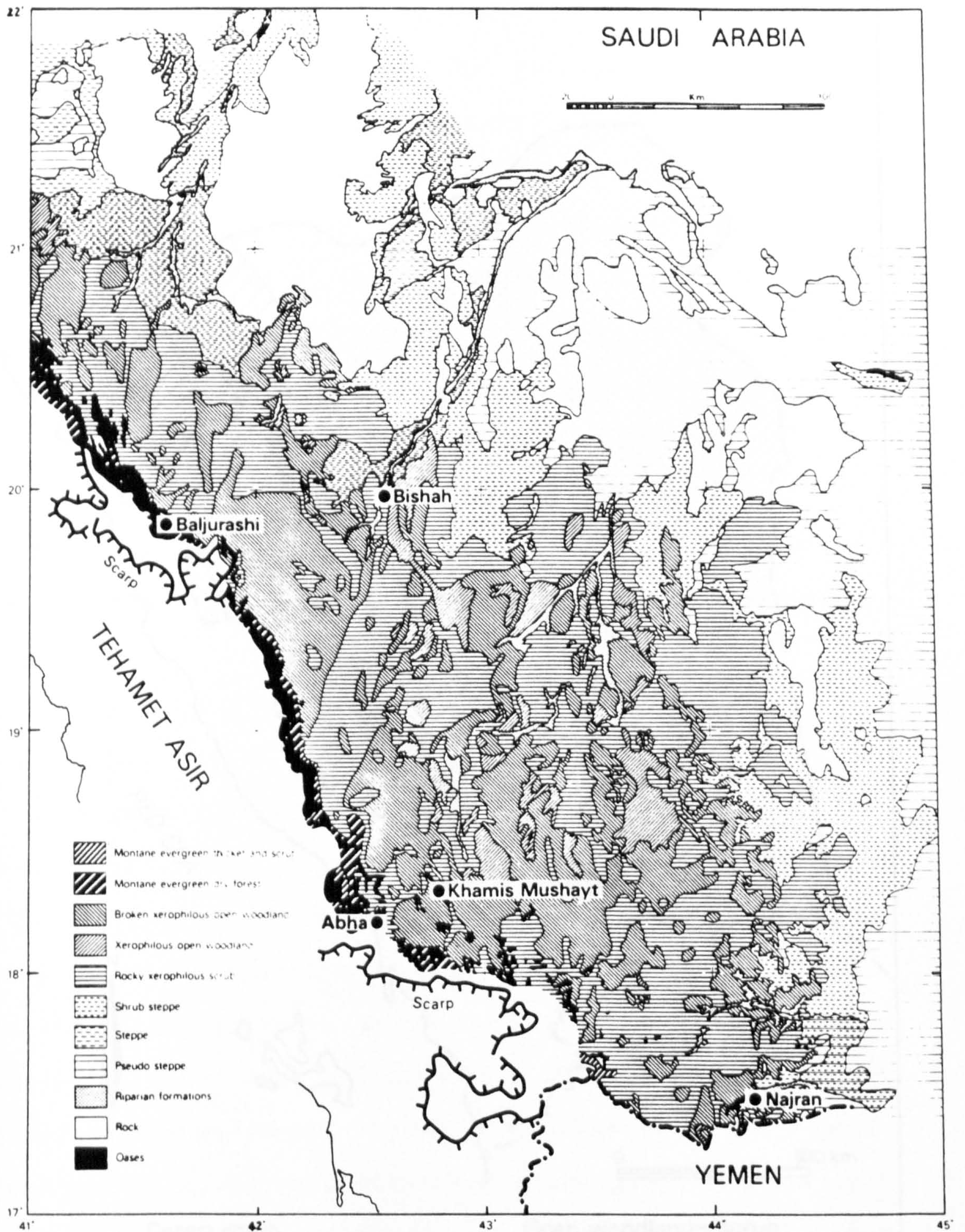
The soils in the southwest region are extremely varied according to topographical zones. The data sources about the soil in this region are limited, although a general description and account of the soil conditions of Asir have been described by Al-Blehed (1982). In general, the soils of the upper mountain wadis are of a coarse texture with concentrations of gravel and sand with much less clay content. The soil which ranges from less than 10 cm to 40 cm deep is yellow to yellow-brown in colour.

In the lower mountains, the soils are of a finer texture than the upper mountain soil, deeper, wider and less stony.

To the east of the Asir mountain range, large valleys come down from the mountains; the soil of these valleys is composed of fine sand, sometimes with saline alluvial materials, laid down in the valley generally to a depth of several metres. Several soil samples taken at a depth of 20 cm at various locations throughout Al-Baha region, have been found to be alkaline. (Finnplanco-Ahmed A.Hajjan Consultants,1985).

5. Vegetation

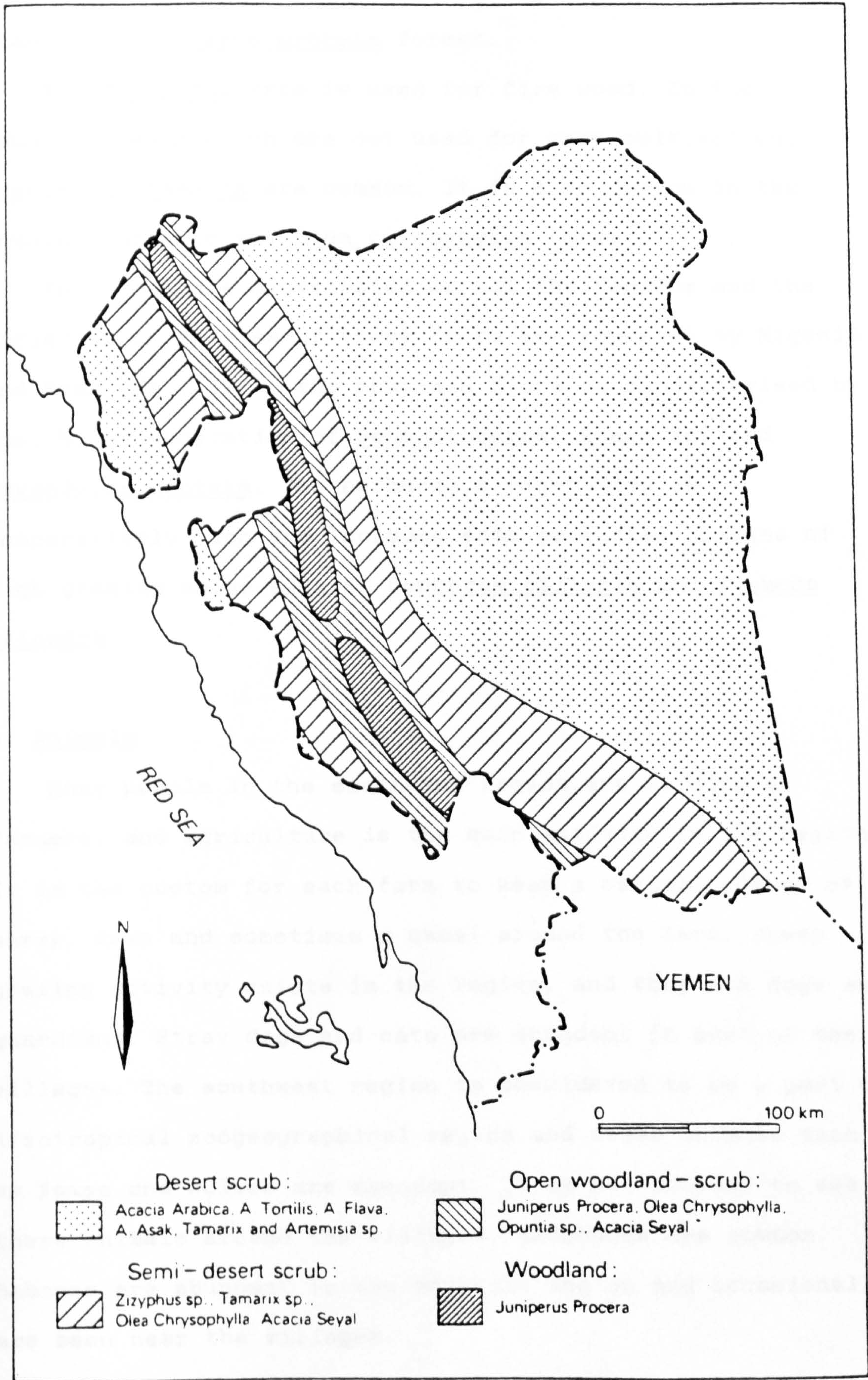
Vegetation zones in Asir were described by Al-Blehed (1982) and are shown in maps 4 & 5. They run from the dry Tahama lowland, where a beach vegetation along the Red Sea coast is replaced by sand dunes association a little further inland in the foothill areas in Tahama region. The xerophilous woodland is the most common. On the upper



Source:Ministry of Agriculture & water,Saudi Arabia
taken from Al-Blehed,A.,1982,P.136

Map 4 Vegetation cover of Asir, east of the main north – south highway (after de Marco)

Map 5 The main Zonation of Vegetation in Asir Region



Source: Al-Blehed, A., 1982, P.138

slopes of the escarpment there are significant natural stands of Juniperus procera forest.

The Juniperus tree is used for fire wood. In the mountain wadis which are not used for crop cultivation, Acacia and Tamarix are common. It is also common in the mountain area to see Olea crysophylla forest.

The flora are distributed on all the regions and the varieties of species of these flora are reported by Migahid and Hammouda (1978); the mountain flora is characterised by low, bushy vegetation of Senecio odrus, Romex sp. and Lavendular dentata. The herbaceous vegetation is comparatively rich and includes some perennial grasses of high grazing value such as Cenchrus ciliaris and Themeda triandra.

6. Animals

Many people in the southwest region are working as farmers, and agriculture is the main activity in the region. It is the custom for each farm to keep a certain number of sheep, cows and sometimes a camel around the farm. Sheep grazing activity exists in the region, and they use dogs as guardians. Stray dogs and cats are abundant in most of the villages. The southwest region is considered to be a part of Afrotropical zoogeographical region and other animals such as foxes and wolves are abundant. It is not unusual to see these animals around the villages. Hedgehogs are common. Baboons are abundant in the mountain region and occasionally are seen near the villages.

TABLE 5 Number of hospitals, beds, primary health care centres, and parasitic disease control centres in the study area (Source: Ministry of Health, annual report, 1984)

Health facilities	Abha (Asir)	Al-Baha	Gizan
Hospitals	7	2	6
Number of beds	928	578	849
Primary Health Care Centres	177	58	75
Malaria Control Centres	4	1	9
Schistosomiasis Control Centres	3	1	1
Leishmania Control Centres	1	1	1

Remarks: Taking the nation as a whole:

there are 2.6 hospital beds per 1000 people.

there are 726 people to each physician.

there is one Primary Health Care Centre to 8250 people.

7. Transportation and Communication Services

Abha city in the Asir mountain range is linked by a network of paved roads with Al-Baha province and Gizan. In fact this is part of the main road from Taif to Gizan through the mountain range.

Another main road connecting the villages in the three provinces is the road from Jeddah to Gizan, passing through the Tihama region near the foot of the mountain, via Mukwah and Mahyel cities where another main road has been constructed to connect between the main roads in the low and highlands.

In addition to the road network, there are regular Saudia flights between the major cities in the region itself and between the major cities in the Kingdom.

Saudi Telecommunication Services including the telephone, telex and telefax are available in all the cities in the provinces.

8. Health Services

The health system in Saudi Arabia is the national health system provided free to everyone.

The Primary Health Care Centres (PHC) are the first step in the health service, followed by provincial hospitals, then specialized hospitals for tertiary health care.

Each PHC is responsible for a certain catchment area, approximately 9000 population per PHC. The duties of the PHC are to provide early diagnosis and treatment for the

patients, children's immunization programme against communicable diseases, and health education.

The staff members of each PHC are:- one physician, one nurse and one pharmacy technician. However, some PHC's provide more services, such as a laboratory service, X-ray and dental hygiene, according to the annual reports of the Ministry of Health. There are 310 PHC's and 15 hospitals with 2355 beds which are distributed in the major cities and villages across the three provinces.

In addition to the previous health services, the Ministry of Health lays a great emphasis on parasitic diseases and, for this purpose, the Ministry have established specialized centres for malaria, schistosomiasis and leishmaniasis control. Table 5 shows the distribution of the health services for each province in the study area.

Other health services are provided by other Government agencies - Ministry of Defence, National Guard and Ministry of the Interior, and their hospitals provide a primary, secondary and third level health care for their staff and other citizens. The University hospitals, in addition to their education and research purposes, contribute to the health services for the local people. School health units provide a primary health care and health education for students, both male and female.

The Private hospitals and dispensaries are also available in the major cities.

9. Population

The 1974 census is the only source of data about the population in Saudi Arabia and, according to that census, the total population of the southwest region was about 1,250,000, Table 6 shows the number of population for each province by sector.

According to the 1974 census, the southwest provinces are the more densely settled parts of Saudi Arabia. They had, at the time of the census, 26.31, 17.39 and 8.5 persons per square kilometre in Gizan, Al-Baha and Asir provinces respectively. Map 6 gives an indication of the regional population density in 1974. The age and sex structure of the population varied between sectors of population in the three provinces of the study area, for example in Abha (Asir) province, Fig.5 shows the percentage of males and females in each age group. The 20-49 year old group of males was less among the rural and nomadic population than in the urban area (see also Table 7).

10. Prevalence of major diseases

The data about the disease prevalence are mainly reported by the Ministry of Health. Most of these data are handicapped by two factors. First, most of the data are general, crude data based upon the clinical diagnosis of physicians. Secondly, there is a lack of personnel well-trained in both epidemiological and biostatistical methods for scientific analysis of these data. Nevertheless, efforts

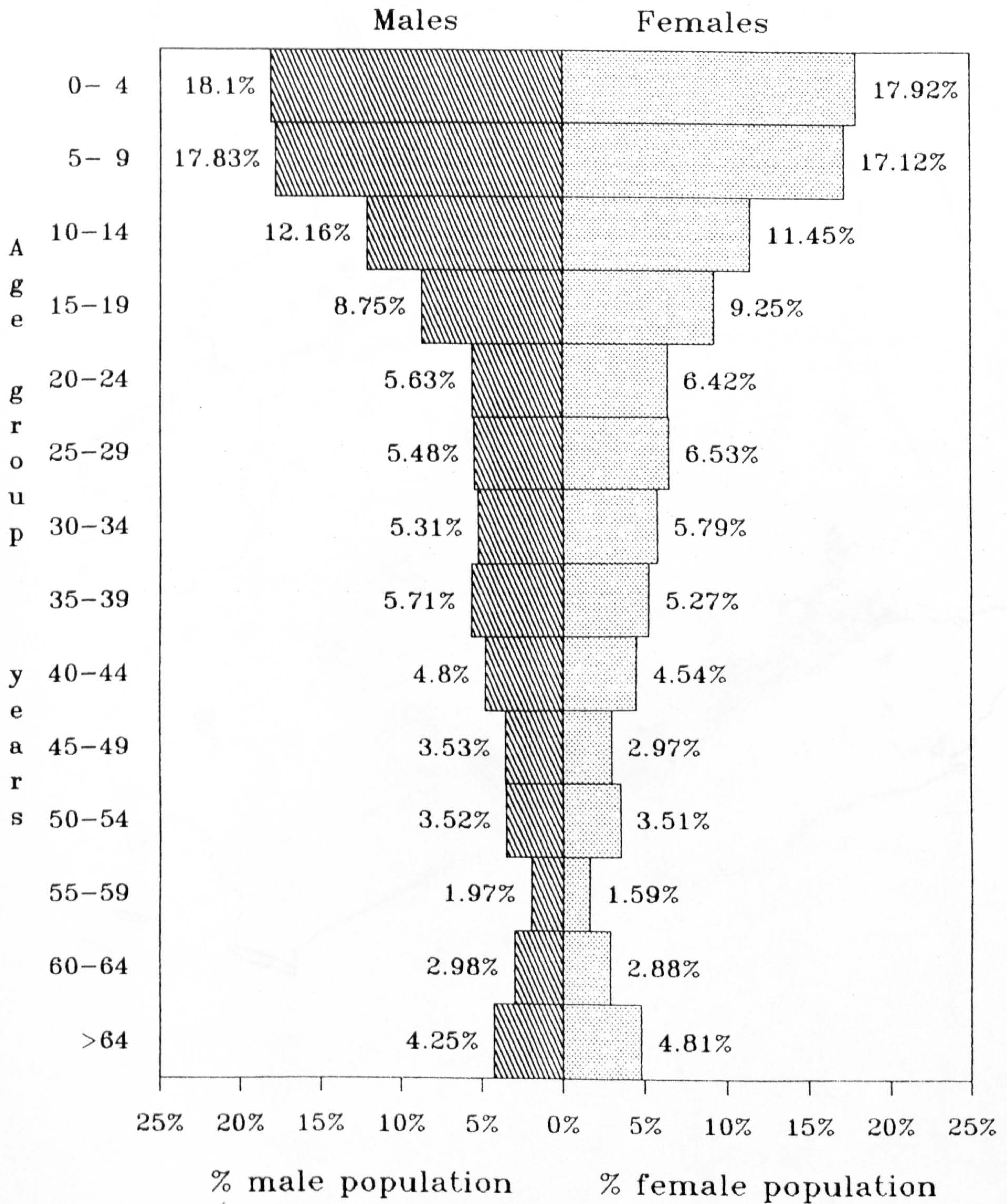
TABLE 6 The urban, rural and nomad population size and population density in the southwest region of Saudi Arabia (Source: 1974 Census)

	Province:		
Population	Asir	Gizan	Al-Baha
Urban	101,970	71,778	5073
Rural	392,274	320,611	151,870
Nomadic	184,435	15,945	28,908
Total	678,679	408,334	185,851
Land area (sq km)	78,437	15,517	10,690
Population per sq km	8.5	26.31	17.39

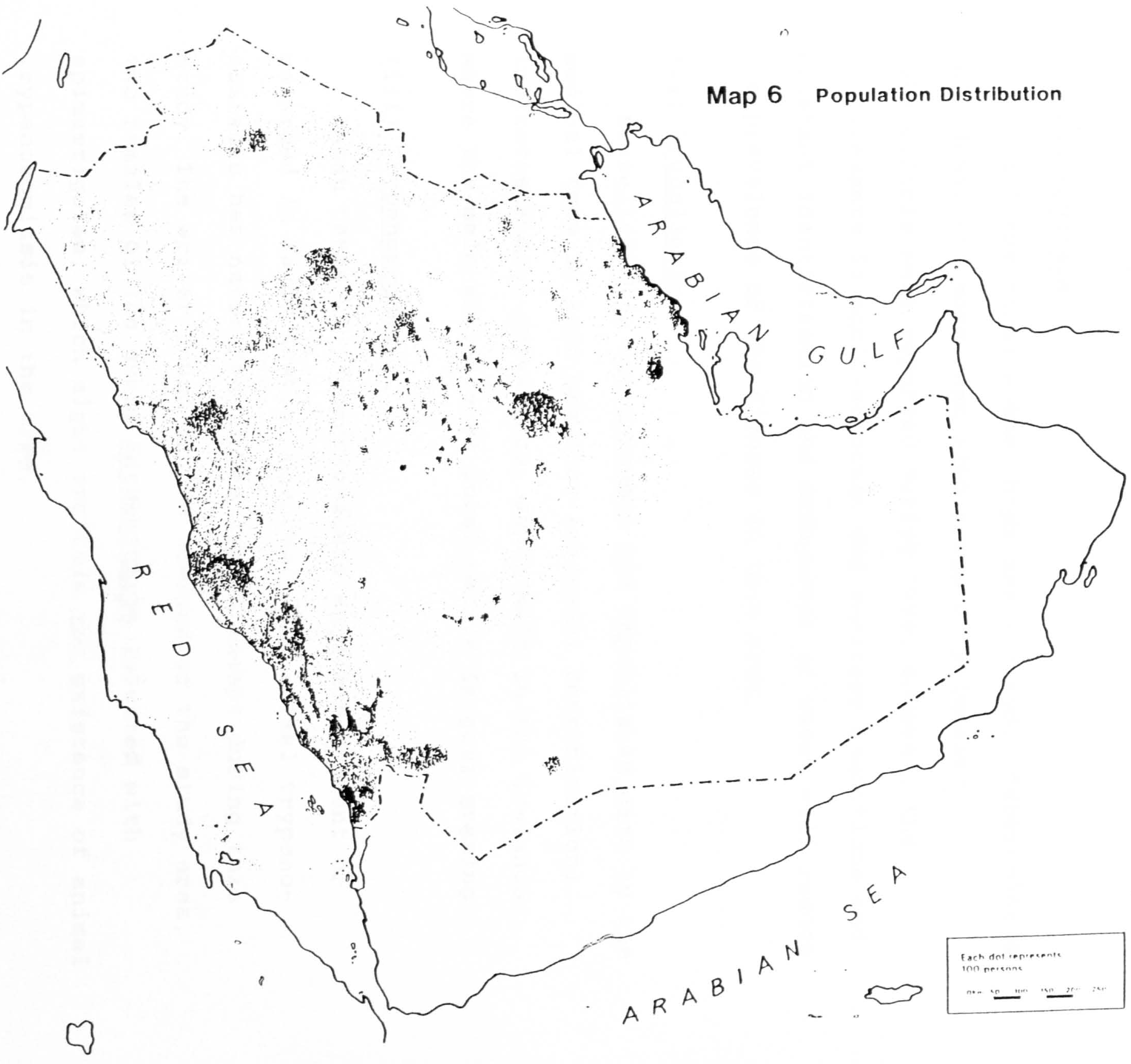
TABLE 7 The age and sex of urban, rural and nomad populations in Asir region (Source: 1974 Census).

Age Group	Urban M	Urban F	Rural M	Rural F	Nomadic M	Nomadic F
0–19	50.8%	59.2%	58.7%	54.7%	57.7%	56.4%
20–49	41.5%	32.4%	27.2%	31.5%	28.9%	31.1%
50+	7.7%	8.4%	14.1%	14.0%	13.4%	12.5%

Fig. 5. Age and sex of the population of Asir Province (source: 1974 Census)



Map 6 Population Distribution



have been made by the Ministry in the last five years to improve the department of biostatistics.

(i) Tuberculosis

In all the study areas (high and lowland), tuberculosis is endemic and more than 1200 cases of pulmonary tuberculosis were reported during 1984. However, the improvements in socio-economic and sanitary conditions and constant identification and management of cases will reduce the prevalence of the disease in this area.

(ii) Brucellosis

Brucellosis is not uncommon and unpublished data by the medical team in Asir province (personal communication) indicates a high prevalence, especially in the lowlands where the main activity of local people is goat grazing.

(iii) Trypanosomiasis

Human cases of trypanosomiasis were not present or recorded in Saudi Arabia; obviously the animal trypanosomiasis has never been investigated except during this study. The writer found in the lowland of the study area, two females of the genus Sergentomyia infected with epimastigotes, which might indicate the existence of animal trypanosomiasis in the area.

(iv) Malaria

Malaria is highly endemic in the Tihama lowlands where it is considered to be the main public health problem.

The mountain range is considered to be free of malaria transmission, except in some of the big valleys where a few cases have been reported.

The data presented in Table 8 show the total cases of malaria which have been reported by the Malaria Control Department from the southwest region.

Table 9 shows the parasite species in Gizan Province where it is seen that Plasmodium falciparum is the dominant species accounting for approximately 97-99% of total cases. Plasmodium malariae and vivax also exist in the area. Mixed infections were reported but they are very few. The main vector is Anopheles arabiensis which breeds throughout the year in shallow, small, unshaded water collections in and around the perennial streams of the foothills, or even in the water of household water tanks and earthen pots in the dry season.

In Gizan Province, the Malaria Department with a team of expert malariologists and the WHO Malariology team are working together to monitor the drug response of P. falciparum, but so far no chloroquine resistant strains have been reported from the country.

The control measures that have been applied in the Kingdom of Saudi Arabia have been essentially the use of chemicals against vector species. In 1984, the vector control measures were changed from residual house spraying with DDT to regular weekly larviciding with Abate(R) on the breeding places. It is applied in combination with simple

TABLE 8 Malaria annual parasite incidence (API) per 1000 people in the Southwest region (1985-1987) (Source: Ministry of Health, Preventive Medicine Department annual report 1988).

Province	Year	Number examined	Number positive	API/1000
Gizan	1985	213,524	12,457	33.2
	1986	250,716	7,848	20.1
	1987	270,057	11,712	30.4
Al-Baha (Galwa)	1985	4,890	54	2.6
	1986	29,671	617	29.8
	1987	30,985	117	6.0
Asir, sectors:				
Abha	1985	9,562	516	1.3
	1986	10,441	658	1.5
	1987	15,030	870	1.9
Mohayel	1985	10,717	251	1.9
	1986	11,337	289	1.9
	1987	17,249	1,648	11.1
Alfrishi (Tahama)	1985	1,805	106	11.1
	1986	4821	240	36.9
	1987	5,326	391	41.6

TABLE 9 Malaria incidence in Gizan province (1982-1984) (Source: Ministry of Health, Preventive Medicine Department annual report, 1985).

	Blood examinations:			*Plasmodium* species:			
Year	Examined	Positive	API	*falciparum*	*vivax*	*malariae*	mixed
1982	95,016	8,908	25.6	8,727	70	108	3
1983	108,833	10,444	29.3	10,158	170	133	3
1984	91,956	7,232	19.8	7,030	104	95	3

TABLE 10 Prevalence of schistosomiasis in the Southwest region (1986-1987) (Source: Ministry of Health, Preventive Medicine Department annual report 1988).

		Schistosoma species:			
Province	Year	*S. haematobium*	*S. mansoni*	mixed	Total
Gizan	1986	1908	0	0	1908
	1987	707	0	0	707
Al-Baha	1986	90	2034	6	2130
	1987	26	1086	1	1113
Asir	1986	814	396	1	1211
	1987	649	715	4	1368
Total		4194	4231	12	8437

site reduction along the valleys of highly populated endemic areas.

Occasionally ultra low volume (ULV) control aerosol spraying with synthetic pyrethroid formulations of dwellings and outdoor resting places of positive villages were employed, in conjunction with mass drug administration.

(v) Schistosomiasis

Schistosomiasis is endemic in both the Tihama region as well as the mountain region. Table 10 shows the prevalence of the disease in the study area.

Schistosoma haematobium is the main species in the Tihama region while *mansoni* is dominant in the highlands. Nevertheless, mixed infections have been reported from Al-Baha and Asir provinces.

Part 3: Field Activities

This part describes the activities which took place in the field including the general methods. Further technical details of the methods for each activity will be discussed in Chapters IV and V.

1. Collection of Human Isolates

1.1 Cutaneous lesions

The laboratory activities were started at the *Leishmania* centre in Abha. This centre diagnosed and treated CL cases only. The total numbers examined during 1985 and 1986 were

(856) and (681) cases respectively. The monthly distributions of these cases are listed in Figure 11. The peaks of reported cases were in the months of November and December.

The writer joined the *Leishmania* centre for collection in the middle of November 1986, which coincided with the peak of reported cases.

The following additional procedures were added by the writer:

(i) We organized a record card (Fig.6) for each patient which contains most of the important data in relation to each patient. This was of great help in the follow-up and mapping of cases, and is now a permanent feature of the clinic.

(ii) The culture technique was introduced as routine, as well as the direct smear.

(iii) Two types of media (NNN and Evans' media) were prepared in the laboratory in Abha and distributed to other health centres in the study area.

(iv) Records of culture follow-up were kept in the laboratory from the isolates from patients in the Abha centre and for any isolates from other provinces in the study area.

Two other centres for the collection of human CL isolates were established in Al-Baha province with the collaboration of the Health authority. These were at Kauwrar PHC and the *Leishmania* clinic centre which were responsible

for treating CL cases in this province.

Collection of isolates were made from time to time by the writer. At the beginning of the study I distributed the culture media with the patient cards to these centres. We failed, however, to collect any positive cultures in the early days for two reasons. Firstly, these laboratories were not equipped with suitable incubators and, secondly, the transportation of the inoculated media to our central laboratory in Abha was dependent upon the time available for me to visit the area. After this happened, a collection of isolates was made from time to time by the writer, usually twice each month, and the culture media were transferred on the same day of inoculation to the central laboratory in Abha.

In Gizan province, because the patients with CL are very scattered in different areas, I was unable to collect any isolates from that province. However, Gizan province is highly endemic for VL and many isolates from these cases were collected from the area.

1.2 Visceral leishmaniasis

Cases of VL are diagnosed and treated in the provincial hospitals. It is compulsory to notify all cases of *Leishmania* . One month after starting the culture technique from CL cases in our laboratory in Abha, I started to collect some visceral isolates.

From field visits to the hospitals in the study area, and screening records of each province, I found that the

Fig. 6. Protocol for patients examined

Ref. FORM

Date:	Lab. No.	Culture No.
Name:	Age:	Sex:
Address:	a)Village	e)Amarah
	Phone No.	d)Period of Stay

Nationality: S NS

Family History:

History of Treatment Duration

No. of Lesion:

Site of Lesion:	Nose	Ear	Arm	Forehead
	Lip	Cheek	Legs	Others
Size of Lesion:				
Morphology of Lesion:	Nodular	Ulcerative	N/U	Scar
Smear:	+Ve	−Ve		
Culture:	+Ve	−Ve		Not Done

<u>Treatment</u>
Pentostam Intra Lesional Systemic

majority of cases were diagnosed and treated in Mahayel hospital in the lowlands of Asir province and King Fahad Central Hospital (KFH) in Gizan province. The diagnosis of VL was based on direct smears of bone marrow aspirate, or serological examination by indirect haemagglutination (IHT), commercially available from the Behring Institute, Germany (Cellognost$^{(R)}$ Leishmaniasis) which is available in Gizan province. Smears from spleen and liver are not often made.

Cultures were not available in these hospitals prior to the time of the study. However, I found that some trials of culture had been made during an examination of the laboratory records of the King Fahad Hospital at Gizan, but that they had been stopped due to technical problems of the blood supply.

During my visit to these hospitals I activated the culture technique by supplying them with culture media (for type of culture and details of methods used see Chapter IV). Plates 1 to 3 show patients with Kala-azar in KFH from whom bone marrow samples were collected and cultured.

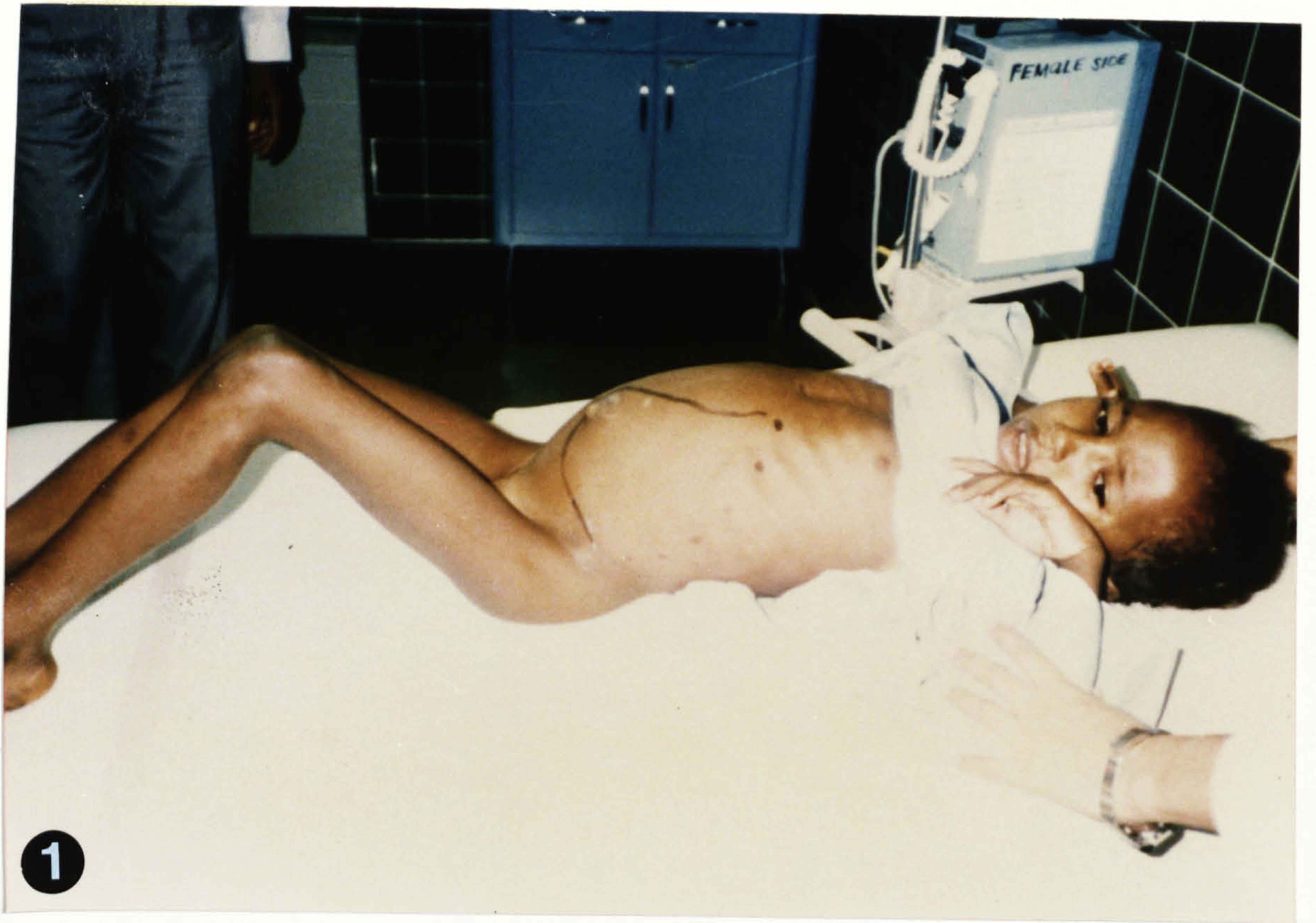
2. <u>Collection of Blood Samples for Serology</u>

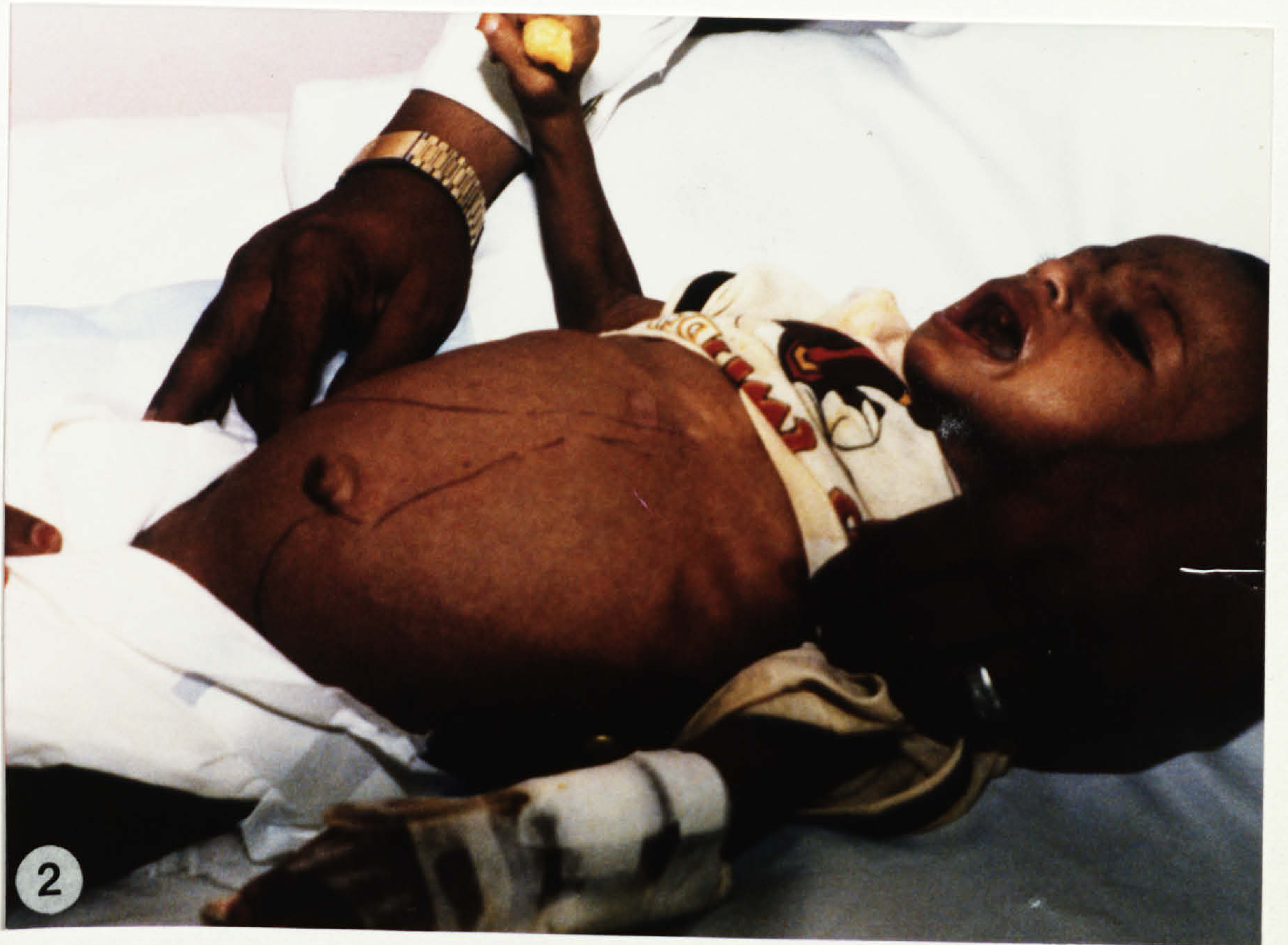
Some 710 blood spots were collected from children under five years old on Whatman No.4 filter paper. The samples collected from the lowlands of the study area, were kept in plastic bags and transferred to the laboratory in Abha. They were kept in the freezer (-20 ^{o}C) until they were transferred to the laboratory of the Department of Medical Protozoology

Photographs of Kala-azar patients

in King Fahad Hospital, Gizan

Plates 1 and 2. Splenomegaly and hepatomegaly in Saudi infants with Kala-azar





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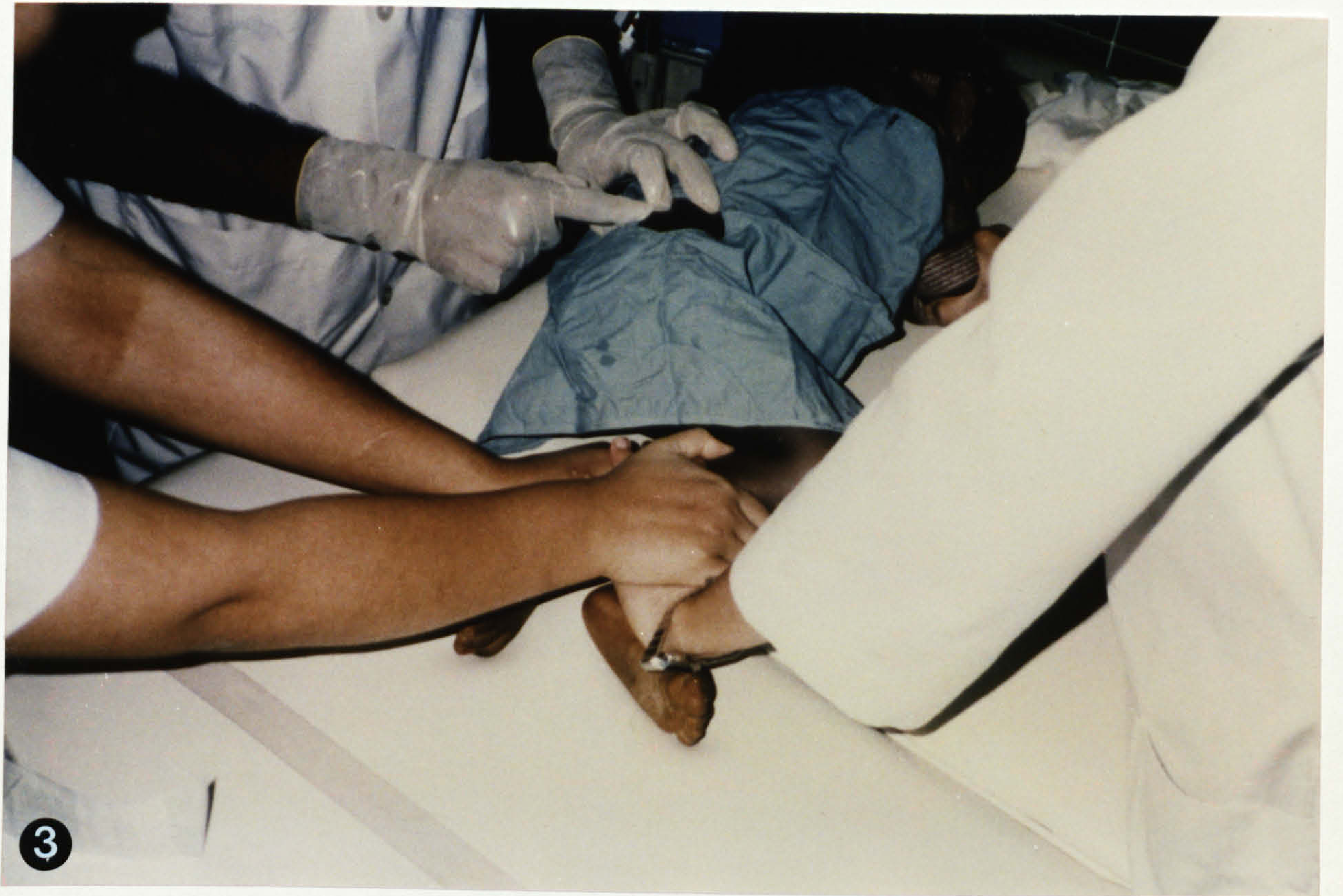
Examination of infant with Kala-azar

Plate 3. A biopsy sample being taken from the iliac crest bone marrow after anaesthetising the skin and periosteum with local anaesthetic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at the LSHTM, London, where they were again kept at $-20^{o}C$ until used.

3. Investigation of Kala-azar Cases

The focal structure of kala-azar in Saudi Arabia is not precisely known. In fact no real attempt has been made previously to investigate and map cases. The collection of samples and their analysis from patients' contacts and the patients' environment have been ignored by the *Leishmania* control department. Therefore in order to improve this type of investigation, I developed a record form (in Arabic and English) for case investigation which has to be filled in by the *Leishmania* team in each province for any kala-azar case. As is shown , the form (Annex 1) covers all aspects of the disease and vector, emphasizing the need to collect samples for further studies. In practice, and after testing the form in the field with the *Leishmania* Control Department, we found that the collection of animals during the case investigation was too difficult for the time being and that only the animals most noticed will be recorded. This is because the *Leishmania* team are not trained in animal trapping and they do not have the facilities for animal examination. The form is in English and Arabic so that it can be understood by the Arabic and non- Arabic workers.

During the period of this study, I participated in more than 20 case investigations by the Asir *Leishmania* team. All the reports from the provinces were sent to the Ministry of Health Leishmania Control Department, Riyadh. By

agreement with the Ministry of Health, all the Asir case reports and samples were kept in our laboratory in Abha. The results of this study will be given in Chapter V. Part 3 (1.4).

4. Reservoir Studies

No information regarding the reservoirs of kala-azar was available. A report from Buttiker *et al*.(1982) and an unpublished report from members of the NLRP (unpublished report, 1983) assumed that canines, or possibly other carnivores, may play a role in kala-azar transmission. In comparison with other countries, we assumed that dogs are the main animals which are implicated. Therefore this study was conducted to find out whether dogs are the reservoir of kala-azar in the study area or not.

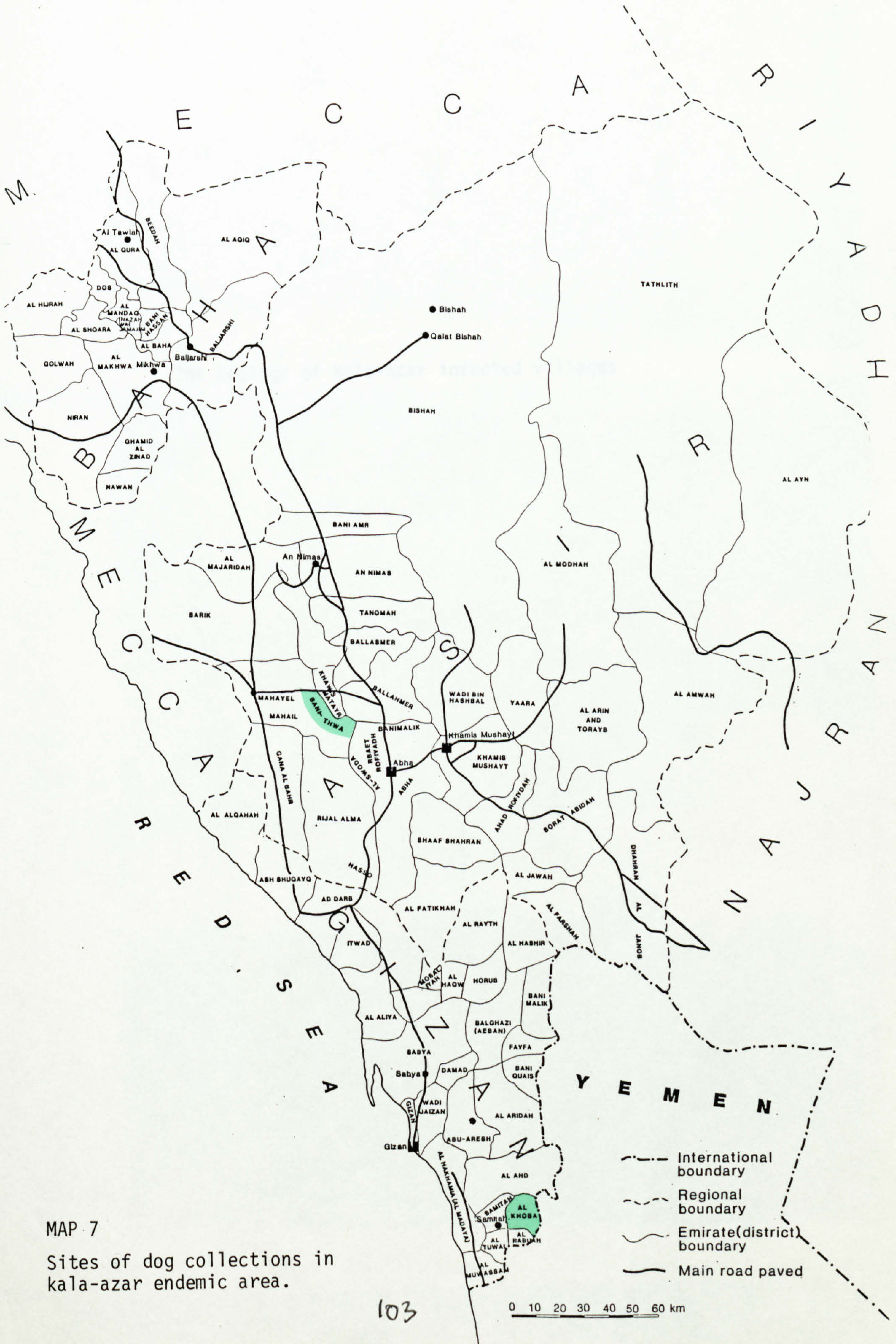
4.1 Place of collection

The reservoir study was carried out in two areas, one in Asir province, Mahayel sector (Bani-Thwa) and the other in Gizan province, Al-Khoba sector. Both areas are highly endemic for kala-azar (see maps 7 and 11).

(i) Bani-Thwa, Mahayel Sector, Asir province

This area is located in the foothills of Tahamet Asir. It is 65 km from Abha city, at an altitude of 480m above sea level and the average rainfall is about 250 mm.

The area consists of eight villages, located on the edge of a valley running from the escarpment of the Sarwait mountain. The inhabitants work mainly in agriculture, goat and sheep grazing. The houses are built of stone and cement.



MAP 7

Sites of dog collections in kala-azar endemic area.

The ecology of Kala-azar infected villages

Plate 4. View of a Kala-azar endemic village in the lowlands of Asir Province. This village is located at the edge of a seasonal river (Wadi) with relative vegetation including trees (commonly Acacia and Tamarix spp)

Plate 5. Deserted houses in a Kala-azar endemic village in Mahayel area. These houses are built from stone and covered inside with mud. The newer white buildings are those which are currently occupied.

Plate 6. Conical thatch covered structures are a common type of house in the Al-Khoba (Gizan) and other littoral parts of the endemic areas of the Southwest provinces.

4

5

6

(ii) Al-Khoba area

This area is located in the Gizan sector near the border of Yemen. It is about 400 km from the first study area. Al-Khoba town is located on the edge of a valley called wadi Khalb. The population of this town with the villages around it is about 11755 inhabitants (unpublished data from the Malaria Control Programme. The houses are mainly huts, but a few are cement houses. Plates 4 to 6 show the type of houses in Mahayel and Al-Khoba respectively.

4.2 Time of study

This study was carried out in two separate parts. The first one was carried out in the Mahayel sector, Tahamet Asir, between 15th and 19th February 1987. The second was carried out between 22nd and 26th March 1987 in the Al-Khoba area, Gizan province. A whole week's preparation period was needed for the field work in each centre.

4.3 Collection of feral dogs

Feral dogs were collected by shooting with M50 gas rifles which were provided by a marksman from the Abha and Gizan Municipalities. The medicament used was suxamethonium (Scoline)(R) which is a muscle relaxant. The dogs were caught twice a day, in the early morning and in the afternoon 2 hours before sunset.

Protection for the team was as follows:-

- vaccination against rabies

- education about the dangers of handling feral dogs
- early arrangements with the local hospitals for any unexpected incident

The team was divided into two groups, one for hunting the dogs and the other for receiving and dissecting them. The hunting team was composed of two riflemen with cars for shooting the animals, three labourers provided with cars for collecting them and taking them to the field laboratory.

The dogs reached the laboratory alive, but were unconscious and most often died or else were euthanised with ether. All of them were dissected within one hour of death. The advance agreement of officials and local residents was obtained and they welcomed this type of study and were very helpful to the team.

4.4 Examination of dogs

Facilities were made available in the field laboratories in the study areas. In the Mahayel area, a tent with electricity and protected from outside contamination was used as a laboratory (see plate 7). All the chemicals and reagents needed for the study were provided by the Ministry of Health.

In the Al-Khoba area, the malaria station was used as a laboratory.

As mentioned above, each dog was dissected within one hour of its being shot. Once the animals reached the field laboratory, the animal card (place of collection, date, sex,

Investigation of dogs in the field

Plate 7. A tent supplied with electricity used as a field laboratory during the reservoir study in the Mahayel District.

length, height etc) was completed for each dog before dissection and remarks registered on its physical examination.

The following procedures were used:-

1. Impression smears from spleen and liver from all dogs.
2. Biopsy for culture from all spleens and livers.
3. Spleen aspiration from dogs Nos. 43 to 91.
4. Blood spots for serology from all animals.
5. Pieces of liver and spleen were stored in 10% formalin to be used for tissue sections.

I should emphasise that all these procedures were carried out in the field in improvised conditions, away from our central laboratory in Abha.

The details of methods used are given in Chapters III and IV.

5. Entomological Studies

5.1 Selection of fixed trapping stations

Entomological studies were carried out in the Asir Province in order to study the sandfly population, to attempt to incriminate the vectors, their seasonal distribution and other biological aspects.

Two fixed stations, in different ecological regions, were chosen, one in the highlands (Shuhat area) and another in the lowlands (Bani-Thwa area). These two fixed stations

were chosen under my supervision for the following reasons:-

(i) According to the statistical records of the Leishmania Centre in Asir Province, the Shuhat area had a reasonable number of CL cases, while Bani-Thwa had a high prevalence of VL cases. This gave us an opportunity to study the sandfly species which are associated with the prevalence of these two diseases.

(ii) The Ministry of Health sprayed some areas as part of their policy for controlling the disease. The two areas mentioned above, however had never been sprayed and this was not planned during the time of this study.

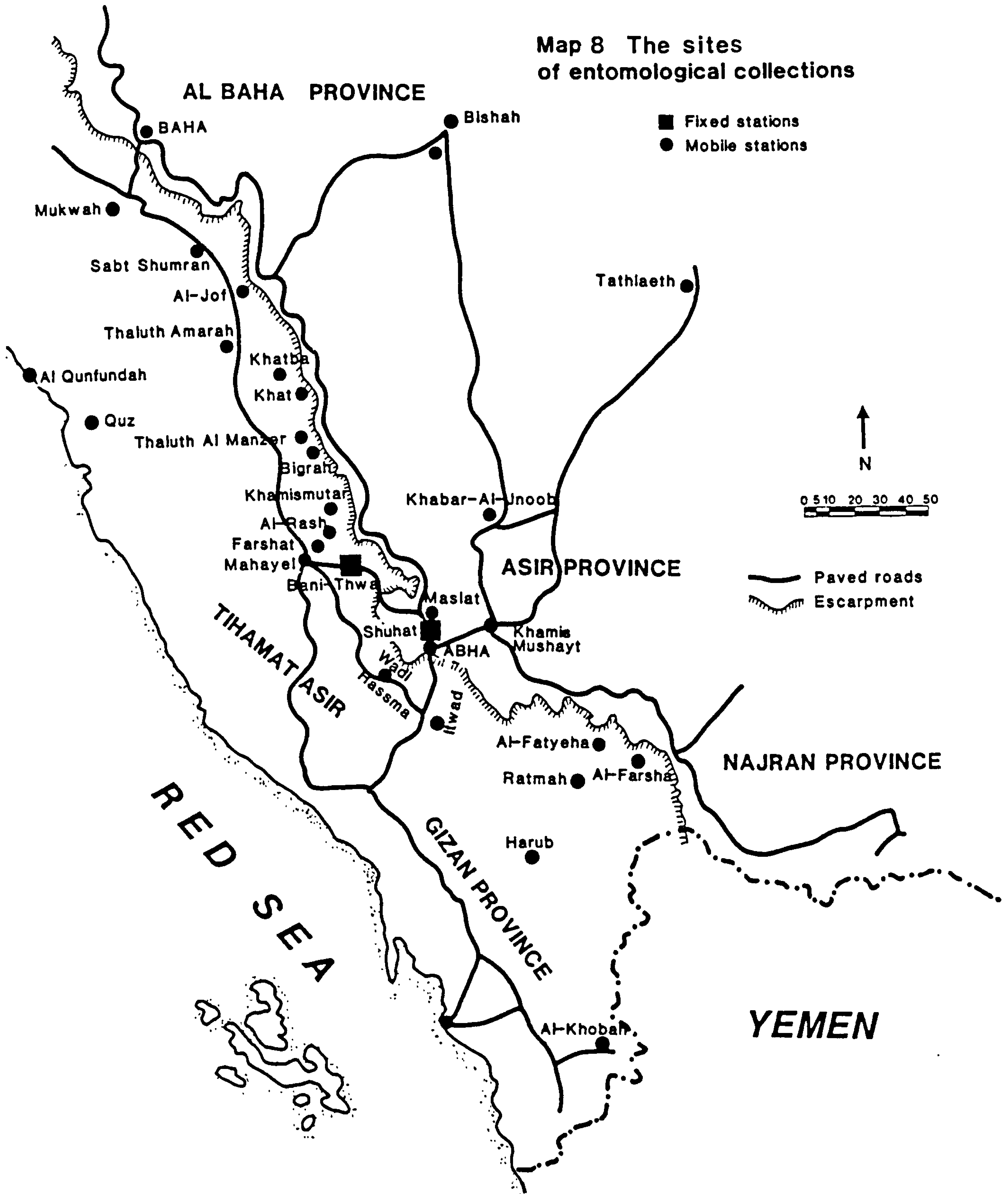
(iii) Both areas are a short distance from Abha city and a good highway provides access to them.

5.2 Description of the fixed stations

(i) Shuhat area (highlands)

As shown in map no.8 this area is about 20 km to the north of Abha City, at an altitude of 2,060 m above sea level. It is located between the hills, with scattered houses and small farms around them. The modern houses are usually built up side by side with the old, traditional style houses which are built of stone mixed with mud (see plate 8).

The inhabitants of this area work in agriculture, growing barley, wheat, sorghum, vegetables and fruit. The water used comes either directly from the collection of rain or from wells.


Map 8 The sites
of entomological collections
Fixed stations
Mobile stations
AL BAHA PROVINCE
BAHA
Bishah
Mukwah
Sabt Shumran
Al-Jof
Tathlaeth
Thaluth Amarah
Khatba
Al Qunfundah
Khat
Quz
Thaluth Al Manzer
N
Bigrah
Khamismutar
Khabar-Al-Jnoob
0 5 10 20 30 40 50
Al-Rash
Farshat
Mahayel
ASIR PROVINCE
Bani-Thwa
Paved roads
Escarpment
Maslat
TIHAMAT ASIR
Shuhat
ABHA
Khamis
Mushayt
Wadi
Hassma
Ilwad
Al-Fatyeha
Ratmah
Al-Farsha
NAJRAN PROVINCE
RED SEA
GIZAN PROVINCE
Harub
Al-Khobah
YEMEN

Ecology of *Phlebotomus sergenti*

Plate 8. The biotope of *Ph.sergenti* in Shuhat area near Abha city in the Asir highlands. Note the farms in the middle distance with houses at their peripheries.

8
87 7 25

There is no meteorological station in the area, the nearest one being in Abha. Figures 3 & 4 show the monthly mean temperature, relative humidity and rainfall at Abha station. However, during our study when we measured the temperature once each week, we did not observe any significant difference from the records at Abha meteorological station.

The flora in Shuhat consists mainly of different species of shrubs, usually to be seen around the farms. Trees such as *Acacia* are scattered around the area. It is not unusual to see sheep and cows in the farms near the houses. The main wild animals such as foxes and wolves are frequently seen in the area.

(ii) Bani-Thwa (lowlands)

This area is the same as the study area of reservoirs which was briefly described in section 4.1 (above). Bani-Thwa is an agricultural area and the main products are sorghum, millet, wheat and barley, in addition to a variety of vegetables. Another activity is that of breeding bees and stock raising such as goats, cows and camels.

The area contains scattered houses built from stone or concrete and a few huts still exist there. The people usually sleep outside the houses in the yards.

The weather is hot during the whole year in this area and is very similar to the Kwash area where there is a meterological station (see Figures 3 and 4).

5.3 <u>Method of collection</u>

Three sites in each fixed station were chosen for the collection of sandflies, old houses about one km away from each other.

Sandflies were collected by two methods.

(a) Sticky traps, waxed paper (42 x 64cm) coated with castor oil and stapled onto wood, placed horizontally approximately 20 cm from the ground at an angle of 90° to the wall (see plate 9).

(b) Light traps were modified as shown in plate 10. American (CDC) light traps were supported by wooden frames and the netting changed to a fine sandfly net. The traps were fixed equally on both sides (indoor and outdoor). Altogether 120 sticky traps and four light traps were used weekly for each fixed station.

The traps were fixed once a week for each station. They were set one hour before sunset and collected early the following morning and transported to our laboratory in Abha.

The timetable of this activity was as follows:

(i) Every Saturday evening, traps were set in the Shuhat area and collected at least half an hour before sunrise on the following day.

(ii) Every Monday evening, traps were set in the Bani-Thwa area and collected on Tuesday morning.

The details of the laboratory work will be given in Chapters III and IV.

The sandfly collections from the fixed stations were

Sandfly traps

Plate 9. The siting of sticky traps in deserted houses was one of the main methods used for sandfly collections.

Plate 10. Modified American CDC light traps were supported by wooden frames and the netting was changed to a fine sandfly net.

9

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87 7 20

carried out in December 1986 and continued monthly, without interruption to the end of December 1987. The contribution from the *Leishmania* team in Abha was a great help.

5.4 Additional sandfly collections ("mobile stations")

(i) Night catches

Night catches were carried out using human bait by the malaria team who had been involved in this activity for years and were therefore assumed to be immune. However, the night catches from fixed villages were mainly carried out by the malaria team from the Mahayel Malaria Station as a a routine monthly collection. The catches were made on one outdoor bait, but indoor baits were occasionally used where possible.

The night catches commenced at 6pm with one bait and the collector and continued every hour, with 15 minute intervals in every hour until 6am the following morning. While the bait was the same person for the whole night, the collector changed shifts with another person in the middle of the night. The locations of collection sites classified as "mobile stations" are shown in map 8.

(ii) From patients' houses during a kala-azar case investigation.

As was mentioned before, any kala-azar case should be investigated by the *Leishmania* team. Collection of sandflies from patient's houses is required in any case investigation. The traps used were sticky traps (indoor and

outdoor) and, on some occasions, light traps were added.

The sites of collection of all the sandfly samples from fixed stations and mobile stations are shown in map 8. The details of dissection and identification will be discussed in Chapter III.

CHAPTER III LABORATORY PROCEDURES IN SAUDI ARABIA

1. Direct Smears from Lesions in Man

Leishmaniasis is diagnosed on a clinical basis over the whole of the Kingdom of Saudi Arabia, with the exception of a few hospitals and some health centres, where poor direct smears may be made for confirmation of the clinical diagnosis. However, in our study area and with the collaboration of the Health Authority, we emphasized the use of the direct smear for routine work for any leishmaniasis case. As a matter of fact, in the Abha *Leishmania* clinic direct smears were done for any patient who came into the clinic, but their method of staining was inaccurate and has now been modified according to the following method which was described by Peters in 1985b.

1.1 Cutaneous lesions

Using a barbed dental broach, the following steps were taken -

Step 1 - the lesion was cleaned with an alcoholic swab

Step 2 - the broach was inserted superficially about 3-5 millimetres into the growing edge of the lesion, parallel with the edge and not directed towards the centre of the lesion.

Step 3 - the broach was twisted and gently withdrawn and mixed with a drop of physiological saline on a microscope slide.

Step 4 - when the slide dries, fix the smear with methanol for 3 minutes.

Step 5 - The slide was flooded with freshly prepared 10% Giemsa stain (the concentrated Giemsa stain diluted 1:10 by distilled water, pH 7.4).

Step 6 - The slide was washed by buffered distilled water.

Step 7 - Examine the slide under X 40 and then oil immersion.

1.2 Bone marrow examination for VL

At the beginning of this study and during the preparation phase, I mentioned in Chapter II that I had visited a hospital in the endemic area and had a chance to see a kala-azar patient. I was able to participate with the medical team while they were taking a bone marrow sample from this patient.

The samples were taken by the paediatrician from the iliac crest of the child as follows:

(i) The biopsy needle was inserted after anaesthetising the skin and periosteum.

(ii) The marrow cells and blood were withdrawn in a sterile syringe.

(iii) Two drops of suspension were placed on a clean microscope slide.

(iv) The drops were smeared, dried and stained by 10% Giemsa stain as mentioned above (1.1, Steps 5 and 6).

(v) At least two microscope slides were prepared from

each patient.

(vi) The rest of the suspension of marrow cells is injected into two bottles of culture media which were supplied by the writer.

(vii) The slides were examined by the pathologist at the laboratory. Review of these slides and discussion with the laboratory specialist were done regularly from time to time. In both hospitals (Maheyel Hospital in Asir Province and the King Fahad Hospital in Gizan Province there are Indian specialists with long experience coming from kala-azar endemic areas. Plate 11 shows a positive bone marrow smear from a child infected with L.donovani sensu lato zymodeme (LON-42)in Mahayel area.

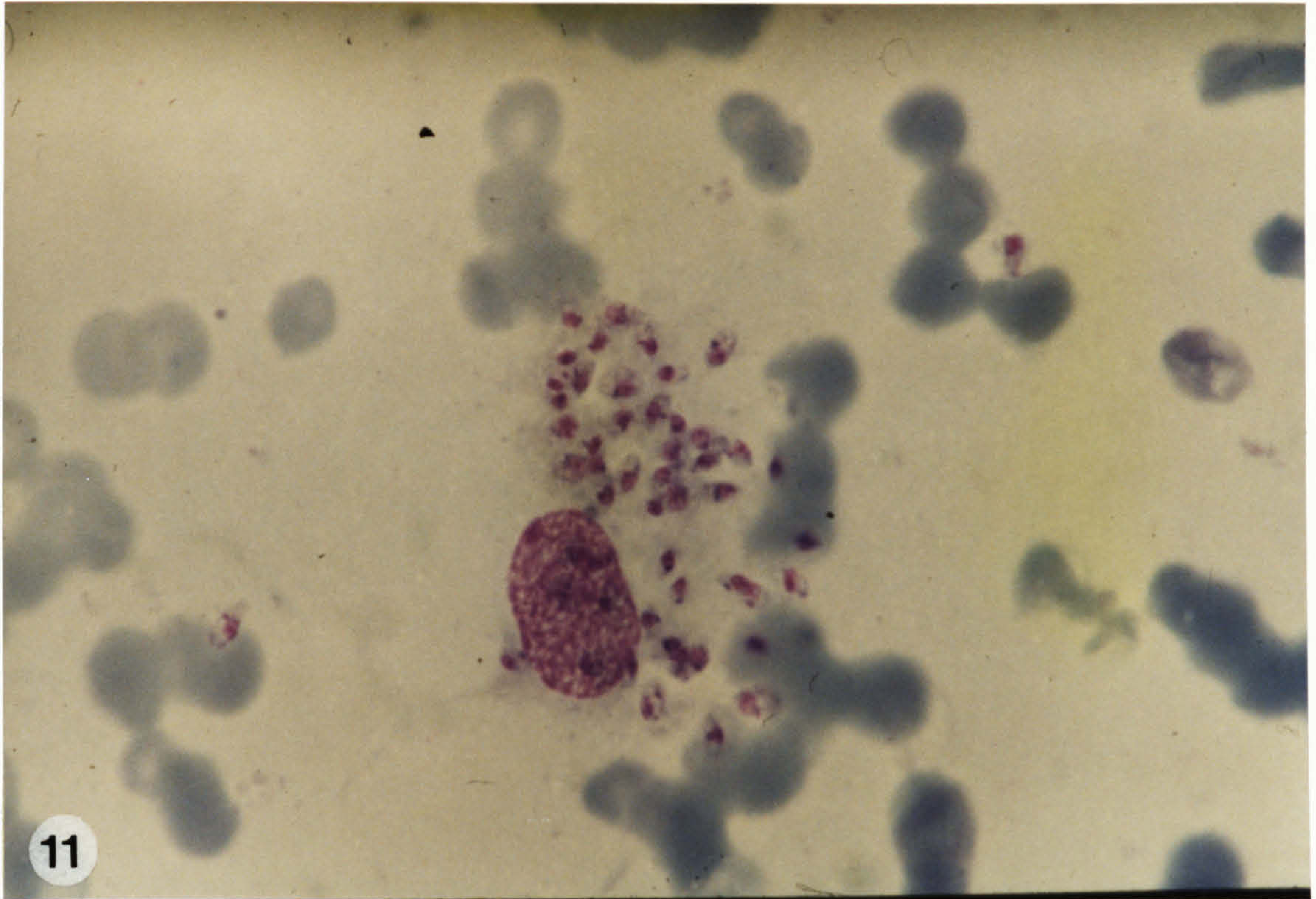
2. Examination of Dogs

2.1 Impression smears

From eighty nine dogs which had been caught in the study area, impression smears were made from both liver and spleen. All the smears were fixed in absolute methanol for three minutes, then air dried and stained with Giemsa. The staining procedures were carried out in the field in the study area as mentioned in Chapter II. All the slides were carefully examined in our laboratory in Abha city, and then re-examined in the laboratory of Medical Protoozology at the London School of Hygiene & Tropical Medicine.

Amastigotes in human bone marrow

Plate 11. Amastigotes seen in the Giemsa stained marrow preparation from a patient infected with Leishmania donovani sensu lato zymodeme (LON-42) (x 1600)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2.2 Tissue sections

A piece of liver and spleen from each dog was preserved in 10% formalin a day after we returned from the field. All the specimens were processed for tissue section at the laboratory of Asir General Hospital. Three slides were prepared from each sample and the rest of the tissue was embedded in paraffin and kept as a block for later use. One slide from each tissue, both liver and spleen, was stained with Giemsa, and other slides were kept to be processed by the immunoperoxidase technique as mentioned later in Chapter IV.

3. Culture

3.1 Culture media used

3.1.1 Media for initial isolation

Referring to what has been discussed in Chapter I, the initial isolation in culture is the most critical step and it is almost impossible to predict which medium is going to be best suited for the growth, especially in the initial isolation. However, two media were used (NNN media and Evans' modified Tobie's media) and I should mention that we used sheep's blood to replace rabbit blood and horse blood in both media because it was the only available blood in our laboratory.

(i) NNN medium (Nicolle 1908)

A. Solid phase

Agar (plain non-nutrient)	1.4g
Defibrinated rabbit blood	25% v/v
NaCl	0.6g
Distilled water	90 ml

B. Liquid phase

This consists of the water that condenses at the bottom of the slopes. 0.2 ml of physiological saline or RPM 1640 was used as overlay.

The solid phase was prepared by melting the agar base in the distilled water by heat with continuous stirring (heat magnetic stirrer was used). The molten agar was transferred to the flask and sterilised by autoclaving the flask at 121°C for 15 min. Cool to approximately 50°C. Heat inactivated (56 C for 30 min) defibrinated sheep's blood was then added to the moltent agar base. 2 ml was sloped in bijoux bottles and kept at 4°C.

The sheep's blood was obtained by bleeding a sheep from the animal house at the College of Medicine, Abha, under completely sterile conditions.

(ii) Evans' modified Tobie's medium (Evans *et al*.1984).

A. Solid phase

Beef extract (Oxoid-Lab-Lemco 129)	3 grams
Bacteriological peptone (Oxoid 137)	5 grams
NaCl	8 grams
Purified agar	20 grams
Distilled water	1000 ml

The above mixture, except the blood, was heated with continuous stirring by a magnetic stirrer until all the ingredients were melted in the distilled water, then sterilized by autoclave at 121°C for 15 min and cooled to approximately 56°C. 15% (v/v) of fresh, defibrinated heat inactivated (56°C for 30 min) sheep's blood was added to the autoclaved mixture. Gentamycin at a concentration of 50 ug/ml was added after gentle shaking of the flask. The medium was dispensed into sterile bijoux tubes (2 ml approximately for each tube), then placed in a sloping position until the agar had solidified and kept at 4°C. (All the prepared media were used within one month).

B. Liquid phase. Proline balanced salts solution (PBSS) (Taylor and Baker, 1978).

KCl	40 mg
$Na_2HPO_4.2H_2O$	6 mg
K_2PO_4	6 mg
$CaCl_2.7H_2O$	18.5 mg
$MgSO_4.6H_2O$	10 mg
$MgCl_2.H_2O$	10 mg
NaCl	800 mg
L-proline	100 mg

phenol red - quantum sufficient.

distilled water to 1000ml.

The above ingredients were dissolved in 750 ml

distilled water and the pH adjusted to 7.2 with solid Tris (tris hydroxymethyl aminomethane), and then the mixture was made up to a volume of 1000 ml and autoclaved at 121°C for 15 min. The solution was dispensed into 100 ml bottles and stored at 4°C. For initial isolation we added 0.1 - 0.2 ml to each bijou bottle of blood agar.

3.1.2 Transport medium

Sloppy Evans' medium (Evans et al. 1984)

Proline balanced salts solution (PBSS)	80 ml
Bacteriological peptone (Oxoid)	0.1 g
Beef extract (Oxoid, Lab-Lemco)	0.03 g
Washed packed horse blood cells	10 ml
Heat-inactivated foetal calf serum	10 ml
Agar (Oxoid, purified)	0.3 g

Preparation of this medium was as follows:

a. The blood cells were centrifuged at 3000g for 10 min (aseptically collected defibrinated blood).

b. Supernatant was discarded and the packed cells were washed twice by resuspension in an equal volume of PBSS and centrifuged.

c. Except the packed cells and foetal calf serum, the above ingredients were mixed together and sterilised by autoclaving at 121°C for 15 min.

d. Cool to about 50°C when the packed cells and foetal calf serum were then added.

e. Mix well and dispense the molten agar into suitable culture bottles.

Note: Dr.Evans kindly prepared and supplied this medium for the field work.

3.1.3 Bulk growth medium

MEM:FCS:EBLB medium (Evans *et al*.1984)

Autoclavable MEM medium (Gibco)	100 ml
Foetal calf serum (heat inactivated)	10 ml
Evan's blood lysate broth (EBLB)	5 ml
$NaHCO_3$ solution (7.5% w/v)	3 ml

This liquid medium was used for mass culture and the EBLB was replaced by Pseudo;EBLB (Evans 1987) as follows:

Tryptose (Oxoid 147)	1.50 g
Casein hydrolysate (Oxoid 141)	1.00 g
Liver digest (Oxoid L27)	1.00 g
KH_2PO_4	0.68 g
NaOH	0.17 g
Distilled water	100 ml

Final pH adjusted to 7.4 by 1m NaOH

The solid ingredients were dissolved in the distilled water and sterilised by autoclaving at 121°C for 15 min. After cooling, 15 ml of an aseptically prepared horse blood

lysate was added to the above mixture. Other blood sources (human, rabbit, sheep) seem to be equally as good (Evans, 1986, personal communication).

The procedures of preparing the blood lysate are as follows:

a. sediment the blood cells by centrifugation at 3,000 g for 10 min.

b. discard the supernatant

c. resuspend the residual (packed blood cells) twice by resuspension in an equal volume of PBSS, centrifuge again at 3000g for 10 mins and discard the supernatant

d. lyse the washed blood cells by adding an equal volume of sterile distilled water and mixing thoroughly. The medium will be very cloudy.

e. Centrifuge the cloudy mixture aseptically at 15000g for 30 min.

f. Decant the clear supernatant into suitable bottles and store at $4^{o}C$.

3.2 Method of culture

3.2.1 From cutaneous lesions

All the initial isolations from cutaneous lesions have been isolated in Evans' modified Tobie's medium.

In the beginning of the study, a barbed dental broach was used to probe the edge of the lesion as described previously in Chapter III (1.1), but after negative results from 32 inoculated cultures by this method, another method

was applied.

Biopsy was performed under sterile conditions and a local anaesthetic by using a circular punch of about 2mm diameter to remove a small circle of tissue from the edge of the lesion. However, this technique has been stopped for the following reasons:

a. It was unacceptable for most of the patients.

b. It was time consuming and depended on the availability of the physician to perform it.

Hypodermic needle aspiration was the main method later used to take samples from the patients for culture inoculation. The procedure of this technique was as follows:

(i) Lesion cleaned with 70% ethanol.

(ii) Using 1 ml disposable syringe, 0.2 ml of sterile physiological saline was gently injected into the edge of the lesion.

(iii) The saline and tissue were aspirated, then gently, immediately and aseptically inoculated into the culture medium and incubated at 21°C.

3.2.2 From kala-azar patients

Cultures from kala-azar patients were inoculated in the hospitals where the culture media was supplied to them by the writer regularly, and as mentioned and discussed in section 1.2 of this chapter, a part of the suspension of bone marrow cells was inoculated into the culture medium.

For every suspected kala-azar case, the bone marrow aspiration was performed by the paediatrician, and two bijoux bottles of culture media were inoculated.

The media were the NNN media with condensed water as overlay and NNN overlayed with 0.2 ml of RPM 1640. Other solutions such as physiological saline were tried as overlay for NNN medium. Evans' medium with PBSS as overlay was used mainly for subculture.

The inoculated media were incubated in the laboratory of the hospital where it was collected regularly, and transferred to our laboratory in Abha city for further follow-up.

3.2.3. From dogs

Immediately the dogs which had been shot were brought from the field laboratory, they were cleaned, physically examined and dissected after euthanasia.

Small portions of liver and spleen were inoculated into 4 bottles of culture medium (two for spleen inoculum and two for liver inoculum). In addition to that, another two culture medium bottles were inoculated by needle aspiration from liver and spleen from the dogs caught in Gizan Province.

3.2.4 From sandflies

Dissection of adult female *Phlebotomus* to seek infected females was carried out twice a week in our entomology laboratory in Abha. The details of the sandfly dissection will be mentioned in the next part under Entomological

Studies (Chapter III, 4.2). However, in the dissected female, the stomach was transferred to a sterile fresh drop of saline on a clean microscope slide, covered with a sterile coverslip, and examined under the binocular microscope. Where it was found to be positive, the slide was sloped to one side and the promastigote solution was aspirated with a sterile disposable syringe and inoculated aseptically to the culture medium (the medium used was Evans' modified Tobie's medium as discussed above, overlayed by 0.2 ml of PBSS).

3.3 Culture follow-up

The collected isolates from the study area were incubated in our laboratory in Abha.

Every inoculum was registered in our culture follow-up notebook. The system for registering each inoculum has two opposite pages, one page for the details of the inoculum, eg name, age, sex, place of collection, address, date of culture etc and the other page for culture follow-up.

Each culture was examined 3 days after the date of culture, and a follow-up examination was made at 2 day intervals. The negative cultures were followed up for five weeks before being discarded.

3.4 Culture contamination

Rigorous aseptic measures were taken for every step of culture technique. Nevertheless, contamination of some inocula was inevitable.

Light bacterial contamination was solved by adding 50 ug/ml of Gentamycin to the liquid phase, and after several passages to new media, clean cultures were frequently obtained. Both heavy fungal and bacterial infections of visceral leishmaniasis isolates were inoculated intra-peritoneally into hamsters for biological filtration. Heavily contaminated cultures of cutaneous lesions were discarded.

3.5 Culture transportation to London

Positive cultures were subinoculated into transport medium (Sloppy Evans' medium) for rapid despatch to London where the isolates were mass cultured, beaded and stored in the cryobank for enzyme typing as described in the next chapter (Chapter IV).

4. Entomological Studies

4.1 Recording system for collected sandflies.

From the beginning of this study I realised that a large number of sandflies would be collected in order to meet the purposes which are mentioned in chapter II. Aiming to avoid any complexicity for presenting data, every specimen was recorded in the entomology notebook with a specific code number.

The code numbers were given for each day of collection and differ according to the locality, site of collection and method of trapping. In other words, the indoor sticky trap collections have different numbers from those of outdoor

sticky traps or light traps. Other details such as locality, and meteorological data were recorded for each code number.

Every code number may relate to one sandfly or more; obviously these were kept in order in the microscope slide box and, when identified were designated the same code number.

4.2 Dissection of sandflies

As described in the previous section (Chapter II, Part 3, section 5.3 the traps were placed in the site of collection every evening and collected from the field after dawn on the following day.

4.2.1 Preparation of sandflies for dissection

When the traps were taken to the laboratory from the field, the sandflies in the light traps were transferred by standard mosquito sucking tubes into a plastic container, which the investigator found to be suitable for large numbers of sandflies (plates 12 and 13).

4.2.2 Dissection procedures

(i) The flies were transferred to a relaxing chamber and killed by ether vapour, then placed on the stage of the dissecting microscope and separated into males and females.

(ii) Each female was placed in a drop of sterile physiological saline on a microscope slide. The specimen was arranged with the ventral surface of the abdomen facing upwards. The head was cut off and transferred to another slide.

Preparation of sandflies for dissection

Plate 12. The sandflies in the light traps were transferred with a standard mosquito sucking tube.

Plate 13. Plastic containers into which the sandflies were transferred from light traps before being taken in groups to a killing and relaxing chamber for dissection.

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(iii) The integument between the 5th and 6th abdominal segment on both dorsal and ventral surface was nicked with the tip of two dissecting needles and pulled posteriorly with the right needle while the abdomen was held by the left needle.

(iv) The stomach was easily recognized by the presence of malpighian tubules at the junction of the stomach and the hindgut.

(v) The tubules were cut near their bases to facilitate subsequent handling of the stomach which it was transferred into a fresh drop of sterile saline placed on a clean microscope slide, compressed with a cover slip and examined under a binocular microscope.

(vi) Promastigotes, if present, were inoculated into the culture medium as described in section 3.2.4 of this chapter. The last segment was transferred to another slide with the head for mounting.

4.3 Mounting

4.3.1 Mounting medium

The following formula of Berlese medium was used for mounting slides:

Gum arabic, picked lumps	12g
Chloral hydrate crystals	20g
Glacial acetic acid	5ml
50% w/m glucose syrup	5ml
Distilled water	60ml

The mixture was kept for at least one hour on a magnetic stirrer at medium speed, and filtered over glass wool to remove the dust. On some occasions when the turbidity was not completely removed, the suspension was kept for 24 hr at room temperature and filtered again.

4.3.2 <u>Preparation of sandflies for mounting</u>

The sandflies collected by castor oil sticky traps and all other dry sandflies were treated as follows:

Specimen kept in 70% ethanol for 24 hr, then washed 3 times in water, then left for 24 hr in 10% KOH, washed 3 times with water. Specimen then ready for mounting.
The head and terminal segments of dissected females were mounted directly after the dissection.

4.3.3. <u>Mounting procedures</u>

(i) The specimen was placed in a drop of Berlese medium on a microscope slide.

(ii) The head was dissected and placed upside down from the main body. The abdomen was arranged in a dorso-ventral position.

(iii) After drying, more Berlese medium was added and covered with a coverglass.

(iv) The slides were labelled and kept at 37-38 ^{o}C overnight.

(v) The mounted specimen was stored in a microscope slide box in order according to the code number, ready for

identification.

4.4 Sandfly identification

All the sandflies of the genus *Phlebotomus* were identified to the species level, but the sandflies belonging to the genus *Sergentomyia* were reported as totals in this genus without species identification.

Males were identified by external genitalia, while the females were separated by the morphology of spermathaecae and armatures of the pharynx. A key for the *Phlebotomus* species known to be present in Saudi Arabia was made using basically the descriptions and illustrations described by Theodor (1948, 1958), Kirk and Lewis (1946a and b, 1951), Perfiliev (1968), Lewis (1982), Lewis and Büttiker (1980, 1982), Lane (1986), Lane and Al-Taqi (1983). Reference slides of species collected from Saudi Arabia were used side by side with the key to check inaccuracies. (The slides were provided by Dr.R.Lane, Entomology Department, London School of Hygiene and Tropical Medicine). Doubtful specimens were sent to Dr.Lane for checking. Plates 14 to 16 show some of the equipment used in the mounting and identification of sandflies in the Abha laboratory.

5. Experimental Infections in Laboratory Animals

5.1 Animals used

(i) Syrian golden hamsters and (ii) BALB/c mice. These animals were bought from the animal house of The College of Medicine, King Saud University, Riyadh, and bred in the

Mounting and identification of sandflies
in the Abha laboratory

Plates 14 and 15. Equipment and instruments used in the mounting and identification of sandflies in the Abha laboratory.

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Sandflies on sticky traps

Plate 16. Removing sandflies from sticky traps prior to mounting and identification

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animal house of the College of Medicine in Abha.

The hamsters were used to maintain the visceral leishmaniasis isolates as "biological filters" for heavily contaminated cultures and for the primary isolates. Both hamsters and BALB/c mice were used to test their susceptibility to Leishmania tropica isolated from cutaneous lesions in the study area.

5.2 Site of injection

For VL, the hamsters were injected intraperitoneally with 0.1 ml of culture. For CL isolates, the hamsters were injected with 0.05 ml of culture in the footpad. At the same time, the mice were injected with 0.05 ml in the footpad. The other non-inoculated footpad was used as a control. The inoculum of CL was from log phase clean, non-contaminated cultures containing approximately 1×10^{7} promastigotes/ml.

5.3 Follow-up of experimental animals

Injected animals were registered in the animal note book with full details, eg, no. of animal, sex, age, animal mark, no. of inoculated culture and date of inoculation. Infection in animals inoculated by CL culture was assessed by weekly measuring of the footpads, and followed up to 6 months. However, by the end of the field study, the inoculated animals were killed, then direct smears and cultures were made from the footpad, liver and spleen.

CHAPTER IV LABORATORY WORK IN THE MEDICAL PROTOZOOLOGY DEPARTMENT, LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE

1. Isoenzyme Typing

1.1 Leishmania stocks

Sixty six (66) *Leishmania* isolates from the southwest of Saudi Arabia were examined, forty four from patients suffering from CL, seventeen from kala-azar patients, three from dogs and two from sandflies (*Ph.sergenti*).

All the stocks were collected during the field work; for more detailed information about the origin of stocks see Tables 24 and 25 and Maps 10 and 11, Chapter V.

1.2 Reference stocks (marker strains)

The following marker strains were used in these studies for comparison with the newly collected isolates:-

A. *Leishmania tropica*

(i) Zymodeme LON-7 (MHOM/SU/60/OD

(ii) Zymodeme LON-22 (MHOM/SA/00/Stiehl)

(iii) Zymodeme LON-63 (MHOM/SA/83/Giz33)

(iv) Zymodeme LON-10 (MHOM/IL/00/Singer)

B. *Leishmania major*

Zymodeme LON-4 (MHOM/SA/84/Jisha 118)

C. Visceral *Leishmania*

(i) *Leishmania infantum* zymodeme LON-49 (MHOM/TN/80/IPT1)

(ii) *Leishmania donovani* *sensu lato* Zymodeme LON-42 (MHOM/SA/81/Jeddah/KA)

The profile markers were run on every plate and, when required, more markers were added, such as MHOM/IN/80/DD8.

1.3 Mass cultivation

The liquid medium, MEM:FC:EBLB (Evans *et al*., 1984) was used to cultivate the large numbers of promastigotes required for isoenzyme analysis. The cultures were incubated at 21 ^{o}C and examined regularly for assessment of growth. Contaminated culture bottles were discarded.

1.4 Harvesting and cryopreservation

In the log phase of culture growth, the promastigotes were harvested by the method which was described by Evans *et al*. (1984) as follows:

(i) The culture of promastigotes was poured into centrifuge tubes and centrifugated at 3000 g at 4 ^{o}C for 20 minutes.

(ii) The supernatant was discarded and the pellet (packed cells) was resuspended in proline balanced salt solution (PBSS), and centrifuged as mentioned above three times. The final supernatant was discarded.

(iii) The pellet was lysed by adding an equal volume of diluted stabilizer solution. The working formula of the solution was -

1 ml 2mM (EDTA) ethylene diamine tetra-acetic acid

0.026 g 2mM (ACA) E-amino caproic acid

0.03 g 2mM (DTT) dithiothreitol

The solution was diluted 1/100 with distilled water

(iv) The above mixture (lysed packed cells with diluted stabilizer solution) was frozen in liquid nitrogen and thawed three times.

(v) The lysed promastigotes in step (iv) were spun at high speed centrifugation (3000 g for one hour).

(vi) By using an automatic pipette, 18 ul beads were made from the supernatant. The beads were kept in liquid nitrogen - cooled bottles and stored in the nitrogen bank until required.

1.5 Enzymes characterised by electrophoresis

For routine use up to 12 enzymes were characterised. These were:

1.	Alanine amino transferase	(ALAT)	E.C. 2.6.1.2
2.	Aspartate amino transferase	(ASAT)	E.C. 2.6.1.1
3.	Superoxide dismutase	(SOD)	E.C. 1.15.1.1
4.	Esterase	(ES)	E.C.3.1.1.1
5.	Nucleoside hydrolase	(NH)	E.C.3.2.2.1
6.	Mannosephosphate isomerase	(MPI)	E.C.5.3.1.8
7.	Glucose phosphate isomerase	(GPI)	E.C.5.3.1.9
8.	Malate dehydrogenase	(MDH)	E.C.1.1.1.37
9.	6-Phosphogluconate dehydrogenase	(6PGD)	E.C.1.1.1.44
10.	Phosphoglucomutase	(PGM)	E.C.2.7.5.1
11.	Proline iminopeptidase	(PEPd)	E.C.3.4.11.5
12.	Pyruvate kinase	(PK)	E.C.2.7.1.40

1.6 Electrophoresis method

The thin layer starch gel method was used for isoenzyme

analysis. The technique being used for this method was the same as that given in detail by Al-Taqi (1978) which was based on the methods used by Smithies (1955), Warxel and Gulliford (1968), Brewer (1970), Kilgour and Godfrey (1973) and Godfrey and Kilgour (1976). The conditions for electrophoresis and for staining the enzymes were basically as described by Evans *et al*. (1984) and Le Blancq *et al* (1986) with the modification of the buffer as made by Evans (personal communication).(See table 11).

1.6.1 Preparation of thin layer starch gel

(i) Each plate requires approximately 50 ml starch. This is a solution of hydrolysed starch made up at 25 ^{o}C which is above the maker's recommendation for serum electrophoresis (given on the bottle).

(ii) The starch was added gradually to the gel buffer in a round bottomed flask with swirling to avoid lump formation. The starch suspension was dissolved by warming the flask directly over the bunsen flame with vigorous swirling until the solution started to boil. When it was boiling, the flask was removed from the flame and the dissolved air was removed by degassing the solution with an electric vacuum pump.

(iii) Generous amounts of starch solution were poured into the edge of the glass plate (230 x 140 mm) and spread with a quick, smooth action. The plate was then covered with another glass plate and left to set. When the gel had set the plate was placed on the cooling plate for at least 20

TABLE 11 Running conditions for thin-layer starch gel electrophoresis

Enzyme	Tank buffer	pH	Gel-buffer Tank buffer:H_2O	Voltage V/plate( )	Running time (hr)
Aspartate aminotransferase (ASAT) EC 2.6.1.1	0.15 Tris[2] 0.15 Citric acid	9	1:9	300	2½
Alanine aminotransferase (ALAT)	same as ASAT buffer	9	1:9	300	2½
Pyruvate kinase (PK)	same as ASAT buffer	9	1:9	300	2½
6-phosphogluconate dehydro-genase (6PGD)	0.1M Tris 0.1M malic acid 0.1M acetate 0.1M EDTA	7.4*	1:9	300	2½
Estrase (ES)	same as 6PGD buffer	7.4*	1:9	300	2½
Glucose phosphate isomerase (GPI)	same as 6PGD buffer	7.4*	1:9	300	2½
Nucleoside hydrolase (NH)	same as 6PGD buffer	8*	1:5	200	2

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Table 11 (contd.)

Enzyme	Tank buffer	pH	Gel-buffer Tank buffer:H_2O	Voltage V/plate ( )	Running time (hr)
Malate dehydrogenase (MDH)	0.2M Tris 0.01M EDTA 0.0476M Nazcitrate	9.5	2:1	140	4
Superoxide dismutase (SOD)	0.019M NaH_2PO_4 0.081M Na_2HPO_4	7.4	1:9	300	2½
Mannose phosphate isomerase (MPI)	0.1M Tris 0.1M Na $H_2PO_4 \cdot {}^2H_2O$	7.6	1:9	300	2½
Proline iminopeptidase (PEPd)	0.15M Tris 0.1M Na $H_2PO_4 \cdot {}^2H_2O$	8.2	1:9	300	2½
Phosphoglucomutase (PGM)	0.25M Tris 0.055M Boric acid 0.005M EDTA 0.0075M mg acetate	9	1:5	250	2½

Footnotes

1. Dimensions of the starch gel were 1 mm x 135 mm x 215 mm; distance between buffer wicks was 150 mm; origin was 35 mm from cathode end except for SOD where it was 60 mm
2. Tris = Tris (hydroxymethyl) aminomethane
3. EDTA = Ethylene diaminetetra acetic acid

*NaOH = pH adjusted with NaOH

minutes before it was loaded.

1.6.2 Preparation tank

The tank buffer was poured into two cells of the tank equally, the cooling plate was placed in position and covered with melinex. Two pieces of sponge wicks were soaked in the buffer of both anode and cathode sides.

1.6.3. Sample application

(i) Samples and the marker strains were taken from the nitrogen bank and placed in order in microtitre plate wells. When the samples were thawed, the microtitre plate was placed on an ice box during the samples' application.

(ii) The slots for the samples were made with a metal slot marker (8 slots). This was positioned about two thirds of the way along the gel except for SOD enzyme which was near the middle.

(iii) Using fine forceps, 0.9 ml lengths of single twist cotton threads (cotton threads were prepared in stock until required) were placed in the lysate.

(iv) The saturated threads were removed from the microtitre plate wells and placed across the slot in a straight position (the thread was tucked into the surface of the gel with fine forceps).

1.6.4 Setting up the electrophoresis

(i) The loaded plate was then transferred on to the cooling plate. A thin glass plate was placed across the middle of the gel plate. The wet sponge wicks were placed

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against the thin glass plate with one end of each wick in the buffer. Finally, the thick glass plate was put in.

(ii) The tank was covered with the lid and the power pack was turned on and adjusted to the appropriate voltage for a certain time (Table 11).

1.6.5 Application of the Developer

(i) The composition of the developers used for each enzyme is given in Table 12.

(ii) Developer buffers and other stable ingredients were checked so as to be available in proper time before they were used. Some ingredients were already prepared and in stock, frozen at -20°C until needed. Others were made up freshly before use.

(iii) The reagents were measured into foil-wrapped, clear bottles and kept cool in an ice bucket. The very sensitive reagents were added about 5 minutes before the complete developer was required.

(iv) After the correct amount of time had passed, the power pack and power point were switched off. The glass plates and sponge wicks were then removed.

(v) To protect the molten agar developer from spreading , the rectangular plastic frame was placed on the plate when it was needed. Some enzymes, eg ALAT, ASAT and ES were monitored by using filter paper saturated in the developer.

(vi) The remaining sensitive chemicals such as MTT, PMS, NBT etc were added to the developer ingredients which were prepared in step (iii) and quickly mixed with the specified

TABLE 12 Staining conditions

Enzyme	Developer buffer	Additional ions	Coenzyme[1]	Linking[2] enzyme	Substrate	Visualization method
Aspartate amino transferase (ASAT)	0.1M PO_4 pH 7.4 6 ml	-	NADH 5 mg	MDH 10 µl	α-ketoglutarate 12 mg aspartic acid 20 mg	filter paper UV
Alanine amino transferase (ALAT)	0.1M PO_4 pH 7.4 6 ml	-	NADH 5 mg	MDH 10 µl	α-ketoglutarate 12 mg L-alanine 80 mg	filter paper UV
Pyruvate kinase (PK)	0.3 Tris/HCl pH 7.4 13.3 ml	0.1 $MgCl_2$ 4ml 1 M KCl 3ml Fructose 1,6 diphosphate 100 mg	ADP(15mg.ml^{-1}) 2.7 ml NADH 5 mg	LDH 10 µl	Phosphoenol pyruvate 22 mg	filter paper UV
Superoxide dismutase (SOD)	0.1M PO_4 pH 8.0 18.8 ml		EDTA (200 mM) 0.2 ml Riboflavin 0.6 mg		NBT (5mg.ml^{-1}) 1 ml	Agar 20 ml (incubator at 37°C in dark for 1 hour)
Malate dehydrogenase (MDH)	0.3 Tris/HCl pH 8.0 12 ml	-	NAD (10 mg/ml) 0.3 ml		L-malate 1M (neutralised with NaOH) 2 ml	MTT 2 ml PMS 1 ml Agar 20 ml dist. H_2O 7.9 ml
-Phosphogluconate dehydrogenase (6PGD)	0.3M Tris/HCL pH 8.0 12 ml	$MgCl_2$ 0.1M 0.5 ml	NADP 0.6 ml	-	6-phospho-gluconate 10 mg/ml 1 ml	MTT 2 ml PMS 1 ml Agar 20 ml

TABLE 12 (contd). Staining conditions

Enzyme	Developer buffer	Additional ions	Coenzyme	Linking enzyme	Substrate	Visualization method
Phosphoglucomutase (PGM)	0.3 Tris/HCl pH 7.4 12 ml	$MgCl_2$ 1M 0.5 ml	NADP 0.6 ml	G6PD (100 μ/ml) 100 μl	glucose-6-phosphate + glucose-1,6-diphosphate ($20 mg.ml^{-1}$) 1.5 ml	MTT 2 ml PMS 1 ml Agar 20 ml
Estrase (ES)	0.1 M PO_4 pH 7.4 6 ml				4-methyl umbelliferyl butyrate 1 mg in 200 μl of acetone	filter paper UV
Nucleoside hydrolase (NH)	0.3M Tris/HCl pH 7.0 16 ml			xanthine oxidase 100 μl	inosine 2 mg	MTT 2 ml PMS 1 ml Agar 20 ml
Proline iminopeptidase (PEPd)	0.15 Tris/HCl pH 7.4 14 ml	0.5M $MnCl_2$ 0.4 ml		peroxidase 2 mg aminoacid oxidase 2 mg	L-pheny-Proline 20 mg	3-amino-9-ethyl carbazol 25 mg in 1 ml ethanol Agar 20 ml
Mannose phosphate isomerase (MPI)	0.3M Tris/HCl pH 7.4 12 ml	$MgCl_2$ 1M 0.5 ml	NADP (10 mg/ml) 0.6 ml	GPI 16 μl G6PD 100 μl 200 μl	Mannose-6-phosphate 10 mg	PMS 1 ml MTT 2 ml Agar 20 ml
Glucose phosphate isomerase (GPI)	0.3M Tris/HCl pH 7.4 13.4 ml	$MgCl_2$ 1M 0.4 ml	NADP 1 ml	G6PD 100 μ/ml 100 μl	D-fructose-6-phosphate (10 mg/ml) 1.6 ml	MTT 2 ml PMS 1 ml Agar 20 ml

TABLE 12 (contd.) Staining conditions

Footnotes:

1. Coenzyme abbreviations as follows:

   (i) NADH = nicotin amide adenine dinucleotide (reduced form)

   (ii) NAD = nicotin amide adenine dinucleotide

   (iii) ADP = adenosine diphosphate

   (iv) EDTA = ethylene diamine tetra acetic acid

2. Linking enzyme:

   malate dehydrogenase (MDH), lactate dehydrogenase (LDH), glucose-6-phosphate dehydrogenase (G6PD), glucose phosphate isomerase (GPI)

3. methyl thiolzolyl tetrazolium (MTT) 5 mg/ml

   phenazine methosulphate (PMS) 2 mg/ml

volume of molten agar (1.2% agar was molten at 60 °C in the water bath) and immediately poured over the starch gel plate.

(vii) The plate was incubated at 37 °C and frequently checked for the progress of developing bands. The bands when developed were photographed.

2. Antibody Testing of Children's Blood Spots

The enzyme linked immunosorbent assay (ELISA) is the most utilised method for seroepidemiological surveys. For this reason it was used as the main method for this survey to assess the degree of endemicity and prevalence of the kala-azar in the lowlands of the study area.

Personal training in this technique was given by Dr. Voller in his laboratory at the Immunological Unit of the Nuffield Institute at the London Zoo.

2.1 Samples

A total of 710 blood spots collected from children living in the lowlands of the study areas as described in Chapter II (Part 3: section 2) were analysed by the ELISA method.

2.2 Sample elution

The samples were punched from the filter papers by using the calibration giving 30 ul by a punching machine, and 8 ul for small spots. The samples were then eluted into freshly prepared PBS solution with 0.05% Tween 200 and all

the samples were made to a final dilution of 1:100. Sample elution took place 24hrs before they were tested.

2.3 Buffer solutions

A. Coating buffer 0.05M carbonate

Na_2CO_3	1.59 g
$NaHCO_3$	2.93 g
NaN_3	0.2 g

Dissolve in one liter of distilled water and pH adjusted to 9.6.

B. Phosphate buffered saline (PBS) (10 x concentration)

NaCl	400 g
KH_2PO_4	10 g
$Na_2PO_4.12H_2O$	145 g
KCl	10 g
Distilled water	5 liters

The solution was prepared as a stock concentrate and required volumes were diluted just prior to use.

C. Incubation buffer

PBS as above + 0.05% of polyoxyethylene sorbitan monolaurate (Tween 20, Sigma).

D. Washing buffer

Same as incubation buffer

2.4 Antigen coating plates

The microplate was coated with sonicated parasites of an L82 Ethiopian strain of *L.donovani*(code L82). The antigen was suspended in carbonate T/bicarbonate buffer. The optimal concentration of the antigen was 1:300 with 4×10^{9} promastigotes/ml.

2.5 The substrates

2 mg tablets of O-phenylene diamine hydrochloride which are soluble in water. They were stored in the dark at $4^{o}C$.

The working formula for each plate was -

4 tablets of O-phenylene diamine

10 ul hydrogen peroxide

12 ml distilled water

2.6 Stopping solution

The stopping solution was 3 M HCl

2.7 Conjugates

Monoclonal antihuman IgG labelled with horseradish peroxidase kept at $-20^{o}C$ for routine use. The checker board titration showed that the optimal dilution of conjugate is 1:3000 diluted in incubation buffer.

2.8 ELISA method

Different methods of ELISA have been developed by

different workers. The method which is described here is the method of Voller et al (1976), Voller et al. (1980).

(i) The coating antigen plates were allowed to proceed overnight for sensitization.

(ii) Plates washed three times with washing buffer.

(iii) 100 µl of 1/500 dilution samples in incubation buffer were added in duplicate. The control negative and positives were added to each plate with the same dilution of samples. This sample dilution was found to be the optimal dilution after checker board titration.

(iv) The plates were covered and incubated in the incubator at 37 ^{o}C for one hour. To prevent any dryness, the plates were kept in a humid chamber.

(v) After washing the plates 6 times, the conjugate was added in a 1/3000 dilution (made up in incubation buffer) 100 ul in each well. The plates were taken back to the incubator as mentioned above in (iv) for one hour.

(vi) The plates were washed again as mentioned above, then 100 ul of the substrate was added to each well and kept in the dark for 15 minutes, then the reaction was stopped by adding 50 ul of 3 M HCl to each well. The time of substrate reaction was determined by the experience of Dr.Voller's laboratory.

(vii) The plates were read at 492 nm in the ELISA reader.

(viii) The reading for each sample was registered: (As mentioned above the samples were applied in duplicate and the reading for the samples was the mean optical density for

the well.)

Checker board tests were done for the samples and conjugates to determine the optimal dilutions and, as mentioned above, the checker board tests showed that the optimal dilution to be worked with was 1/500 for the elution and 1/3000 for the conjugate and 1/300 for the antigen.

2.9 The cutoff point

The cutoff point was determined as follows:

a. The mean OD reading was corrected by defining the positive control as 1.0 and running a reference sample on each plate.

$$\text{Corrected OD} = \text{mean OD of sample} \times \frac{1.0}{\text{mean OD of positive}}$$

b. After drawing the distribution of samples under study and the positive control, we found a confidence level of 97% and 100% of sensitivity and specificity. The cutoff point was 0.4.

3. Studies on Direct Agglutination Test (DAT)

A simple and economic direct agglutination test for diagnosis of VL has recently been described by Harith *et al.* (1986). It was decided to take this opportunity to make a direct comparison of the DAT with the well established ELISA test.

3.1 Samples

69 samples out of the 710 children's blood spots and 19

dog's blood spots which had been analysed by the ELISA technique were chosen to be tested again by the DAT. The samples were eluted in ELISA buffer and stored at -20°C until required.

3.2 <u>Antigen preparation for DAT</u>

(i) Promastigotes of <u>Leishmania donovani</u> <u>sensu lato</u> from the Sudan (MHOM/SD/68/SI) were obtained from the cryobank of the Protozoology Department at LSHTM and were grown in bulk growth medium. (MEM:FCS:EBLB medium, Evans <u>et al</u>. 1984).

(ii) In the log phase of culture, the promastigotes were harvested by centrifugation at 4000g for 15 min at 4°C. (The culture bottles were examined before harvesting and any contaminated bottles were excluded).

(iii) The pellet was washed five times by centrifugation at 3200g for 10 min at 4°C in PBSS buffer.

(iv) 4% of trypsin solution (Difco 1/250) in Lock's solution was added to the packed promastigotes in the proportion of 1 volume of packed promastigotes to 20 volumes of trypsin solution and mixed well to resuspend the promastigotes. The suspension was then incubated at 37°C for 45 min, centrifuged at 3200g, 10 min at 4°C and washed five times as in (iii).

(v) The pellet was resuspended in cold Locke's solution to a concentration of 2×10^{8} cells/ml to which an equal volume of cold 2% formaldehyde in Locke's solution was added and left at 4°C for 20 hours.

(vi) The promastigote suspension in step (v) was centrifuged (3200g, 10 min at 4 ^{o}C) and washed twice with cold physiological saline. The pellet was then resuspended to the same volume as in step (v).

(vii) The promastigote suspension was stained with 0.02% (w/v) Coomassie Brilliant Blue R.250 with continuous stirring at a moderate speed on a magnetic stirrer for 90 min.

(viii) The stained parasites were centrifuged and washed twice with physiological saline and the pellet resuspended in physiological saline containing 1% formalin to the same volume as in (v). The suspension was then passed through nylon gauze of 30 um mesh size, and stored at 4 ^{o}C in a bottle covered in silver foil until needed.

3.3 Procedure of DAT

1. The frozen samples of 1:100 eluted blood spots in ELISA incubation buffer were taken out and thawed, then diluted to 1:200 in physiological saline with 1% foetal bovine serum.

2. 50 ul of eluted blood spots 1:200 were placed in V-shaped microtitre plate wells, starting from Row 2 to 12. Wells 1 (A-H) negative control and wells A from 2-12 used as positive controls.

3. Two-fold serial dilutions of samples were made from row A down to row H by adding 50 ul of physiological saline with 1% heat inactivated foetal bovine serum to each well in the plate.

4. 50 ul of stained antigen preparation was added to each well. The plate was then carefully shaken by hand on a level surface for half a minute, covered and left for 18 hours at 21 ^{o}C in the cooling system incubator, where it was then read directly and the reciprocal dilution of each sample was recorded.

4. Examination of Material Collected from Dogs

4.1 Tissue sections stained by immunoperoxidase

The direct examination for *Leishmania* parasites in sections by the conventional staining methods, such as Giemsa stain, may be inconclusive, especially when very small numbers of parasites are present. The immunoperoxidase technique is more sensitive and may allow an unequivocal identification of *Leishmania* amastigotes (Sells and Burton,1981). Because of the scanty parasites in smears from some dogs and in order to have more accurate results, tissue sections from the dogs' livers and spleens were examined by this method.

4.1.1 Samples

Tissue sections from 170 spleen and liver biopsies of 85 dogs were stained by the immunoperoxidase technique and examined for *Leishmania* amastigotes.

4.1.2 Method

The immune peroxidase technique employed was the one

described by Sells and Burton (1981).

A. Antisera

Promastigote rabbit antiserum was supplied by Mr.M.Guy in 20 ul aliquots and stored at -20°C until needed.

B. Buffer

0.05 M Tris saline pH 7.6

Working formula as follows:

Sodium chloride	8.5 g
Tris (hydroxymethyl) aminomethane	6.057 g
Distilled water	1000 ml

The pH was adjusted with 4M HCl to 7.6.

This buffer was prepared fresh and used for washing and reagent diluent.

C. Reagent

30% Hydrogen peroxide

Normal swine serum

Peroxidase conjugate swine anti rabbit serum IgG

3,3 diamino-benzidine tetrahydrochloride (DAB)

These reagents were stored at 4°C.

D. The procedure

(i) Slides to be stained were numbered using a diamond marker.

(ii) Sections were dewaxed in xylene. The slides were placed in a staining jar containing the xylene which was changed every 2 minutes 3 times.

(iii) Sections were washed from xylene by absolute alcohol three times and brought to water.

(iv) The sections were transferred to the humid slide chamber and incubated in 0.3% H_2O_2/methanol for 30 minutes and rinsed in water.

(v) Sections were incubated with 1:20 normal swine serum for 30 minutes.

(vi) The swine serum was tipped off and the sections were incubated with 1:120 rabbit anti Leishmania serum for 30 minutes.

(vii) The sections were washed in 3 changes of Tris saline buffer for a total of 20 minutes.

(viii) The sections were incubated in a humid slide chamber with 1:20 peroxidase conjugated swine anti-rabbit serum IgG for 30 min.

(ix) The sections were washed as in step (vii).

(x) The substrate (DAB) solution was prepared under precautions measured as follows:

5 mg DAB was dissolved in 10 ml of buffer and immediately before use, 20 ul 30% H_2O_2 was added to the DAB solution.

(xi) Sections were incubated in the DAB solution for 7 min in the dark, then rinsed thoroughly in tap water.

(xii) Sections were lightly counterstained in haematoxylin for 2 min, dehydrated in alcohol, cleared in xylene and mounted in DPX.

4.2 Blood spot analysis by ELISA

88 blood spots collected from dogs were examined by the ELISA technique using the same method described in the

children's seroepidemiological survey. The coating antigen was L82 Ethiopian *L.donovani sensu lato* with concentration 10 mg/ml and the conjugate used was rabbit antidog IgG peroxidase (Miles) at a concentration of 1:1,000. Positive controls were either *L.infantum* (Italian dog) or *L.infantum* (French dog). The negative dog was a British laboratory animal used for non-infectious work. The optimal dilution of blood spot elution was 1:800 and the cutoff point was determined to be 0.5. The ELISA value from 0 up to 0.5 was considered to be negative, while the value from 0.51 upwards was considered to be positive.

CHAPTER V RESULTS

Part 1: Records of cases in Saudi Arabia 1985 - 1987

1. Visceral Leishmaniasis

1.1 Prevalence and incidence

A total of 724 kala-azar cases were reported by the Ministry of Health in the last three years. The number of reported cases was increased from 188 cases in 1985 to 288 in 1987 (see Table 13). These cases were reported from hospitals and diagnosed parasitologically or clinically. The increase in reported cases may be attributed to improvement of diagnosis (see Annex 18).

The real attack rate of the disease among the children is not known due to lack of precise data and absence of proper population census. However, by using the available data the incidence rate of kala-azar in 10,000 children aged up to 15 years was calculated in two main endemic areas, the Asir lowland and the Gizan province. As shown in Table 14 and Figure 7 the incidence in Gizan province increased from 3.8 per 10,000 cases in 1985 to 9.7 per 10,000 in 1987. The decrease of the incidence of disease in the Asir lowland from 12.6 in 1985 to 3.7 in 1987 is remarkable and the accuracy of case reports, misdiagnosis, malaria spraying campaign and the Mahayel Municipality Environment Protection Department spraying activities may explain the decline of cases.

TABLE 13 Regional distribution of visceral leishmaniasis cases in Saudi Arabia (1985-1987)

	Province:						
Year	Gizan	Asir	Baha	Western	Eastern	Riyadh	Total
1985	90	98	0	0	0	0	188
1986	181	61	2	3*	1	0	248
1987	243	31	11	0	2+	1	288
Total	514	190	13	3	3	1	724

Notes:

* These were introduced cases from Gizan and/or from Yemen

\+ One of these was an introduced case from Pakistan

TABLE 14 Annual kala-azar incidence per 10,000 children up to the age of fifteen, in southwest Saudi Arabia

	Province:					
	Asir (Lowlands)			Gizan		
Year	1985	1986	1987	1985	1986	1987
Total population	161460	167111	172960	488847	505957	523665
Population at risk	77500	80213	83021	234647	242859	251359
Total reported cases	98	61	31	90	181	243
No. cases/10,000	12.6	7.6	3.7	3.8	7.5	9.7

Notes:

1. The figure for the population in Asir (Lowlands) was based on the malaria survey (unpublished data, 1984) corrected by the geometric method as follows:

   Adjusted population size = 1984 census value $(1 + 0.035)^n$

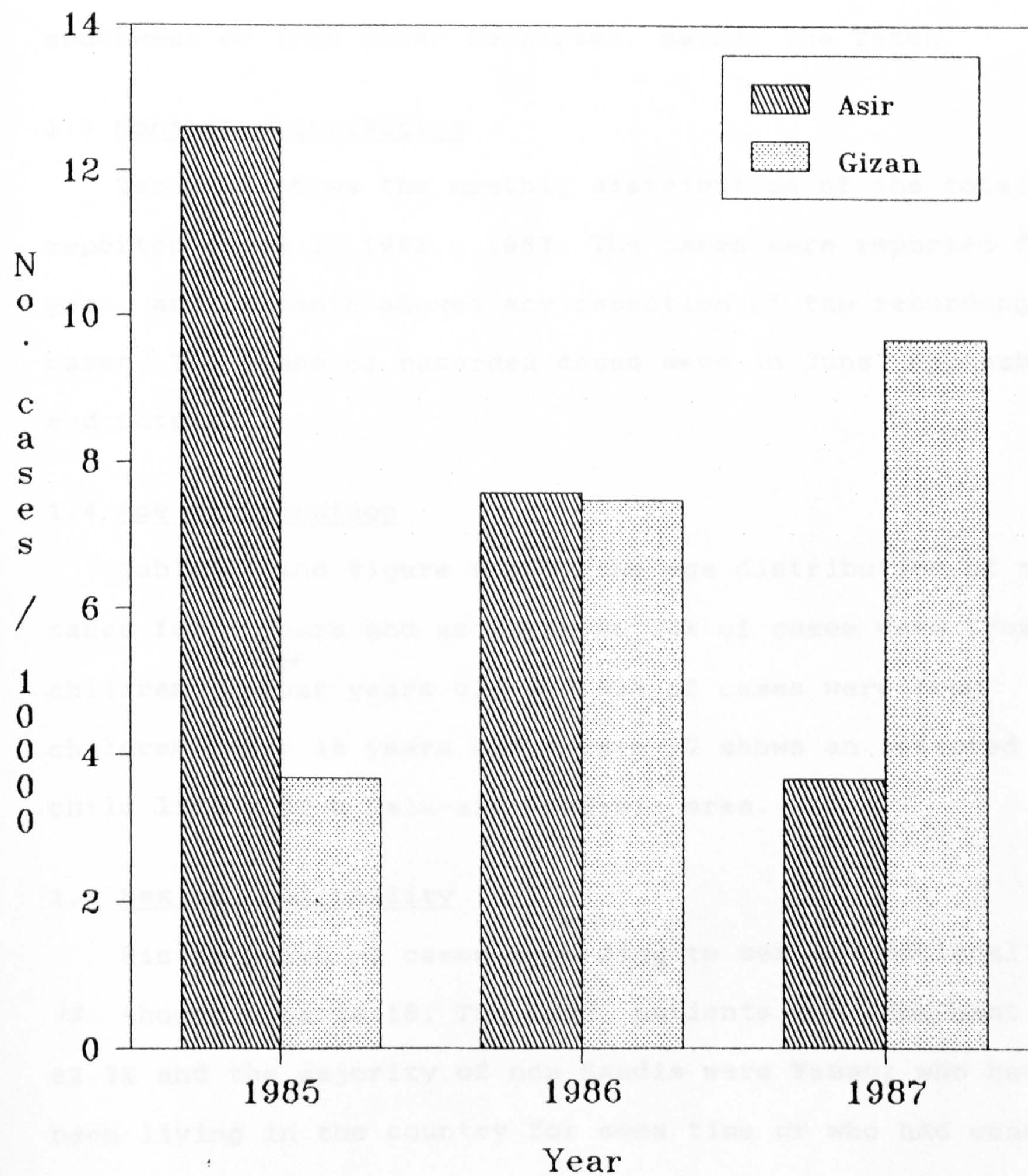
   where n = the number of years since the census was taken, and the natural rate of increase (crude birth rate (0.042) - crude death rate (0.007)) = 0.035 (Kenzo Tange and Urtec, 1976).

2. The population quoted for Gizan is the population of the rural area, which is 82% of the total population according to the 1974 Census. The values were corrected as mentioned above.

3. In both provinces the population at risk was assumed to be those people under the age of 15, living in the lowland rural areas; which according to the 1974 Census comprise 48% of the total population.

4. Few cases were reported in Baha Province and the size of the population at risk is unknown.

Fig. 7. Annual kala-azar incidence per 10,000 children up to 15 years of age in southwest Saudi Arabia (1985-1987)



1.2 Regional distribution

Table 13 shows the geographical distribution of the cases and as seen the majority of cases were reported from the Gizan and Asir provinces; few cases were reported from the Baha and western provinces.

The cases reported from other regions, eg, the Eastern and Riyadh provinces, originated from the lowlands of the southwest or from other countries, mainly the Yemen.

1.3 Monthly distribution

Table 15 shows the monthly distribution of the total reported cases in 1985 - 1987. The cases were reported for a year, and no month showed any cessation of the recording of cases. The peaks of recorded cases were in June, September and October.

1.4 Age distribution

Table 17 and Figure 8 show the age distribution of total cases for 3 years and as is shown 76% of cases were from children under four years old and 95% of cases were from children under 15 years old. Plate 17 shows an infected child living in a Kala-azar endemic area.

1.5 Sex and nationality

Distribution of cases according to sex and nationality is shown in Table 16. The Saudi patients were dominant with 82.7% and the majority of non Saudis were Yemeni who had been living in the country for some time or who had come to Saudi to seek treatment.

TABLE 15 Monthly distribution of visceral leishmaniasis in Saudi Arabia (1985-1987)

Month	1985 No.	1985 (%)	1986 No.	1986 (%)	1987 No.	1987 (%)	Total No.	Total (%)
Jan	15	(8.0)	16	(6.5)	16	(5.6)	47	(6.5)
Feb	7	(3.7)	18	(7.3)	17	(5.9)	42	(5.8)
Mar	11	(5.9)	29	(11.7)	17	(5.9)	57	(7.9)
Apr	3	(1.6)	21	(8.5)	36	(12.5)	60	(8.3)
May	12	(6.4)	9	(3.6)	17	(5.9)	38	(5.3)
Jun	15	(8.0)	37	(14.9)	34	(11.8)	86	(11.9)
Jul	17	(9.0)	21	(8.5)	28	(9.2)	66	(9.1)
Aug	15	(8.0)	17	(6.8)	20	(6.9)	52	(7.2)
Sep	22	(11.7)	31	(12.5)	23	(8.0)	76	(10.5)
Oct	29	(15.4)	6	(2.4)	22	(7.6)	57	(7.9)
Nov	25	(13.3)	24	(9.7)	26	(9.0)	75	(9.0)
Dec	17	(9.0)	19	(7.7)	32	(11.1)	68	(10.4)
Total	188	(100)	248	(100)	288	(100)	724	(100)

TABLE 16 Nationality and sex of patients with visceral leishmaniasis in Saudi Arabia (1985-1987)

	Nationality		Sex		
Year	Saudi	Other	Male	Female	Total
1985	168	20	107	81	188
1986	192	56	157	91	248
1987	239	49	169	119	288
Total (%)	599 (82.7)	125 (17.3)	433 (59.8)	291 (40.2)	724 (100)

TABLE 17 Age distribution of patients with visceral leishmaniasis in Saudi Arabia (1985-1987)

	Age of patient (years):					
Year	<1	1 - 4	5 - 14	15 - 44	>45	Total
1985	19	119	39	6	5	188
1986	24	167	43	12	2	248
1987	43	178	56	9	2	288
Total (%)	86 (11.9)	464 (64.1)	138 (19.1)	27 (3.7)	9 (1.2)	724 (100)

Fig. 8. The age distribution of visceral leishmaniasis patients in Saudi Arabia (1985–1987, n = 724)

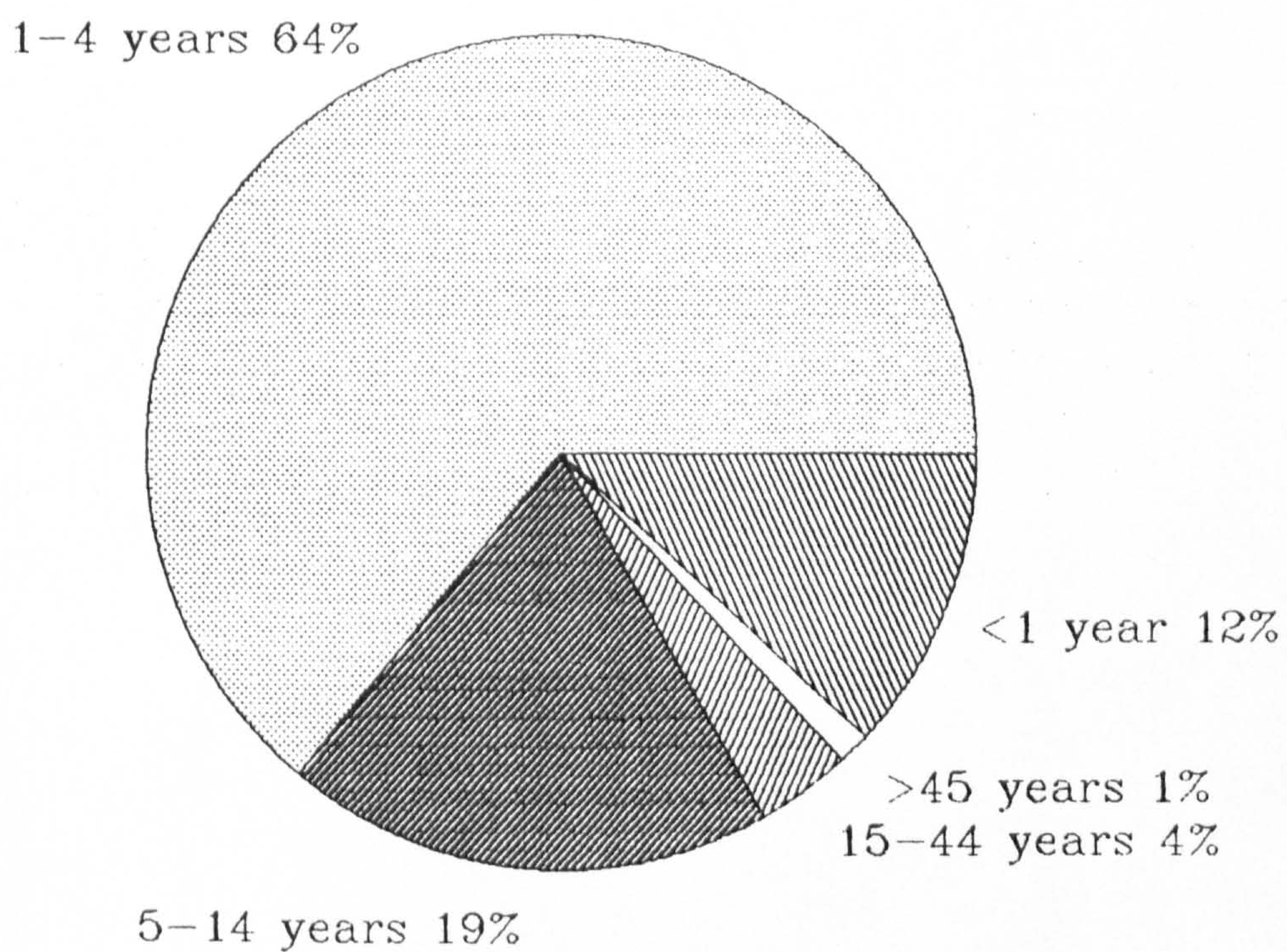


Plate 17. An 18 month old child with splenomegaly and positive bone marrow smear seen in the village of Bani Thwa, Mahayel District.

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2. Cutaneous Leishmaniasis

2.1 Prevalence and incidence

The numbers of cases recorded as CL in the country 1985 - 1987 are shown in Table 18. The increase of cases may be attributed to better recogni tion and diagnosis by local physicians, and this is indicated if these data are compared with early reported cases where there were only 1043 cases in 1978 (see Table 2).

As mentioned in the VL study, the attack rate of the disease is not known and to have an idea about the incidence of the diseases the cases recorded during 1987 from provinces with L.tropica, eg, Asir and Abha provinces, and the Eastern province with L.major were analysed as seen in Table 19. The incidence rates were 12, 38 and 37 per 10,000 of the population. The incidence rate was calculated on the number of cases recorded among the Saudis and the non-Saudi patients were excluded.

The incidence rate of each age group is shown in Table 19 also and as is shown the cases per 10,000 in the age group of less than five years old in the Eastern province L.major area is significantly greater than in the L.tropica area. I should mention that the nationality by age group is not available, but in the children's age group one can assume that the Saudis are higher than the non Saudis and this is best illustrated in the age group 16 - 45 years old which were greater in the Eastern province than in the Asir or Baha provinces, because the Eastern province has more

TABLE 18 Seasonal distribution of cutaneous leishmaniasis in Saudi Arabia (1985-1987)

Month	1985		1986		1987		Total	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Jan	1443	(10.9)	2408	(14.5)	3272	(19.5)	7123	(15.3)
Feb	704	(5.3)	1713	(10.3)	1958	(11.7)	4375	(9.4)
Mar	623	(4.7)	974	(5.9)	1288	(7.7)	2885	(6.2)
Apr	405	(3.1)	455	(2.7)	749	(4.5)	1609	(3.5)
May	437	(3.3)	322	(1.9)	242	(1.4)	1001	(2.1)
Jun	621	(4.7)	367	(2.2)	384	(2.3)	1372	(2.9)
Jul	731	(5.5)	597	(3.6)	563	(3.4)	1891	(4.1)
Aug	587	(4.5)	679	(4.1)	938	(5.6)	2204	(4.7)
Sep	1578	(12.0)	1125	(6.8)	1720	(10.3)	4423	(9.5)
Oct	1943	(14.7)	1504	(9.0)	1452	(8.7)	4899	(10.5)
Nov	2045	(15.5)	2679	(16.1)	1791	(10.7)	6515	(14.0)
Dec	2068	(15.7)	3798	(22.9)	2409	(14.4)	8275	(17.8)
Total	13185	(100)	16621	(100)	16766	(100)	46572	(100)
No. cases per 10,000*	12.5		15.0		15.0			

Year: 1985, 1986, 1987, Total

*Number of cases/10,000 of the population at any age at risk.

TABLE 19 Cutaneous leishmaniasis incidence per 10,000 in L. major areas (Eastern Province) and L. tropica areas (Asir and Baha Provinces)

	L. tropica Areas:		L. major Area:
	Asir Province	Baha Province	Eastern Province
1. Total population	990851	290662	1112550
2. Population at risk	990851	290662	833224
3. Total no. cases			
Saudi	1198	1104	3147
Other	472	334	1553
4. No. cases/10,000 of Saudi population	12	38	37
5. No. cases/10,000 by age			
0 - 5 (17.0%)	23	54	113
6 - 15 (29.3%)	20	66	43
16 - 45 (38.6%)	16	35	59
>45 (15.1%)	5	20	11

Notes:

1. Total population and percentage of age group are based on the 1974 Census mentioned in TABLES 6 and 7 ; using the geometric method and assuming an annual rate of natural increase of 3.5%.

2. The number of cases/10,000 by age group is reported for both Saudi and non-Saudi, because although data are available on the age of patients, no data are available for the nationality of patients in each age group.

3. The population at risk is based on the assumption that the whole population in the L. tropica areas (Asir and Baha Provinces) are at risk. The populations of the big cities, Damman, Al-Khober and Dahran, were considered not to be at risk and so they were excluded from the total population at risk in Eastern Province.

foreign workers than any other parts of the country.

2.2 Seasonal distribution

The seasonal distribution of the country is shown in Figure 9 and shows that the cases start to increase in September and reach their peak in December and January. The data in this figure is the total of three years data. To demonstrate the seasonal distribution in the L.major area (Eastern province) and the L.tropica areas (Asir and Baha provinces), the 1987 cases were taken and analysed monthly as shown in Figure 10. Both species were similar in manner and again the increase of cases started from September to reach their peak in December and January. The L.tropica cases had longer peak periods up to the end of March, while the L. major cases started to decline in March and reached the minimum percentage in May and June.

The seasonal distribution of L.tropica with the seasonal distribution of P.sergenti is shown in Figure 12.

2.3 Age distribution

Table 21 shows the age distribution of the cases reported in 1985 - 1987, and as mentioned previously the majority of cases were among the working age group from 16 - 45 years old. Table 22 gives details of age and sex distribution of the cases reported in 1987.

2.4 Nationality and sex distribution

During the last three years the total of Saudi patients were 55% compared to the non-Saudi patients 45%. Regarding

Fig. 9. Seasonal distribution of cutaneous leishmaniasis in Saudi Arabia (1985–1987)

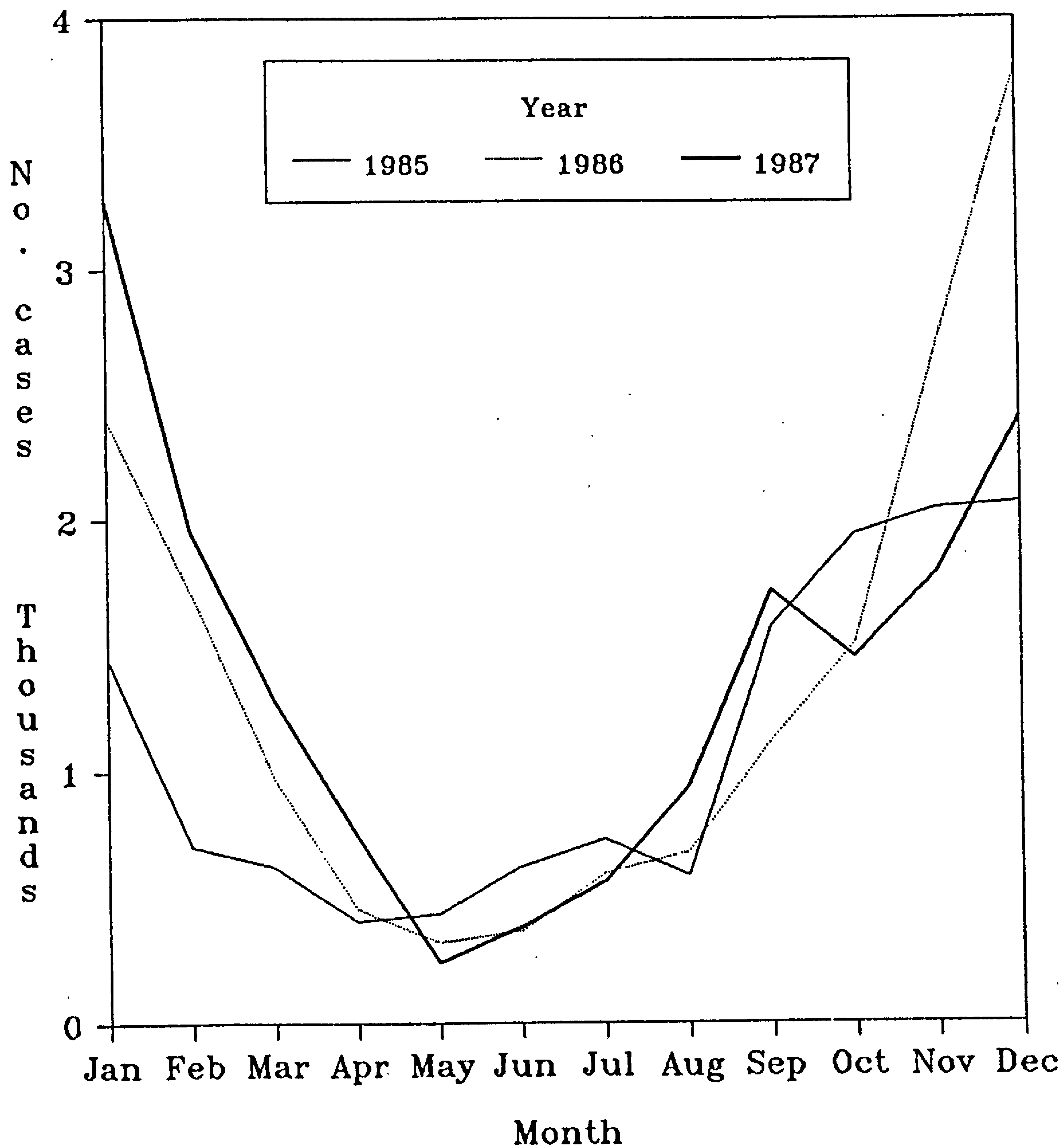

Fig. 10. Seasonal distribution in *L. major* areas (Eastern & Qassim Provinces) and *L. tropica* areas (Asir & Al-Baha Provinces) in 1987

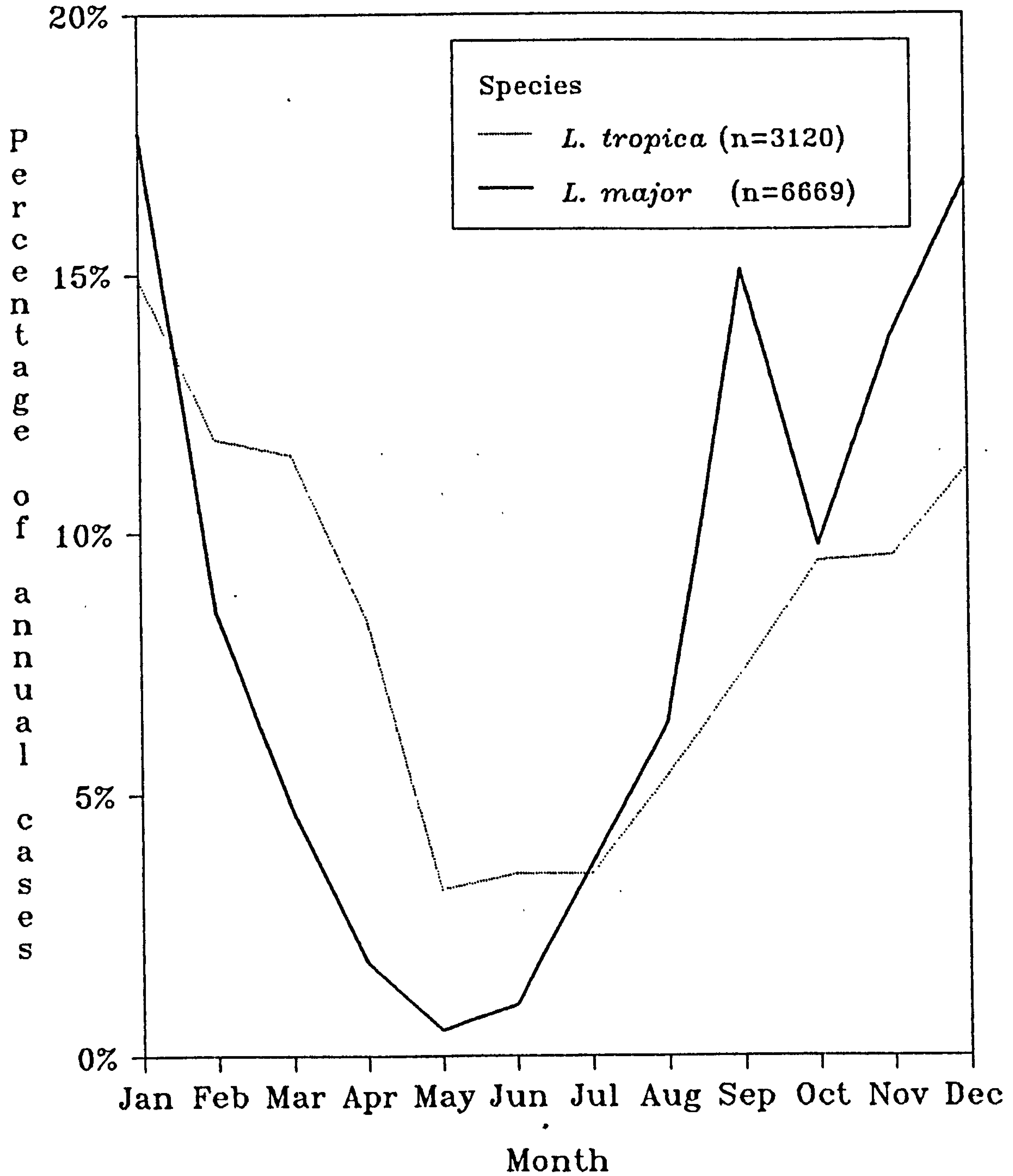

TABLE 20 Nationality and sex of patients with cutaneous leishmaniasis in Saudi Arabia (1985–1987)

	Nationality		Sex		
Year	Saudi	Other	Male	Female	Total
1985	7407	5778	9398	3787	13185
1986	8987	7634	11958	4663	16621
1987	9244	7522	11889	4877	16766
Total (%)	25638 (55)	20934 (45)	33245 (71.4)	13327 (28.6)	46572 (100)

TABLE 21 Age distribution of patients with cutaneous leishmaniasis in Saudi Arabia (1985–1987)

	Age of patient (years)					
Year	<1	1 – 4	5 – 14	15 – 44	>45	Total
1985	529	2097	3189	6709	661	13185
1986	716	3231	3441	8207	1026	16621
1987	755	3264	3703	8286	758	16766
Total (%)	2000 (4.3)	8592 (18.4)	10333 (22.2)	23202 (49.8)	2445 (5.3)	46572 (100)

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TABLE 22 Details of cases of cutaneous leishmaniasis (*L. major* and *L. tropica*) in Saudi Arabia in 1987

Region	Sex		Nationality		Age distribution					
(Province)	M	F	Saudi	Other	<1	1-5	5-15	15-45	>45	Total
Eastern	3024	1676	3147	1553	337	1263	1061	1897	142	4700
Riyadh	3476	821	1690	2607	112	561	640	2796	188	4297
Qassim	1382	390	654	1118	103	287	225	1059	98	1772
Asir	1096	574	1198	472	40	348	584	625	73	1670
Hail	926	516	790	653	85	343	314	643	57	1442
Baha	916	522	1104	334	20	247	565	517	89	1438
Medina & Tabuk	897	274	491	690	52	161	228	636	94	1171
Western	84	41	50	75	2	17	22	72	12	125
Gizan	47	28	61	14	3	21	30	19	2	75
Nijran	20	17	23	14	1	3	18	13	2	37
Qurat	14	9	22	1	0	9	11	2	1	23
Northern	7	9	14	2	0	4	5	7	0	16
Total	9244	7522	11889	4877	755	3264	3703	8286	758	16766

sex distribution, males were dominant with 71.4% (see Table 20).

2.5 <u>Geographical distribution</u>

The case distribution by province is shown in Table 22 which shows that the cases are more concentrated in the Eastern, Central and Al-Qassim provinces which are the areas where the major agriculture and landscape changes were taking place. In the northern Nijran and Gizan provinces the reported cases were much less than any other regions in the country.

Part 2: Studies on Cutaneous Leishmaniasis

1. Epidemiological aspects of CL in the Abha area

The data from the region of Abha are based on the patients who were seen in the Abha *Leishmania* clinic where all the patients were examined clinically. The diagnosis was confirmed in the laboratory by the demonstration of the amastigotes in direct smears.

Data on the seasonal distribution of the disease are based on the three years 1985, 1986 and 1987. The data for 1985 and 1986 were obtained from the records of the *Leishmania* clinic in Abha.

The other epidemiological aspects are based on data obtained during the period from January to December 1987 when 626 patients were positive for *Leishmania* amastigotes in direct smears.

1.1 Geographical distribution

The majority of the patients came from the villages around Abha city, such as Al-Swoda, Banimalik and Ball-Ahmer, see map 9. Few cases were seen in the clinic from lowland areas.

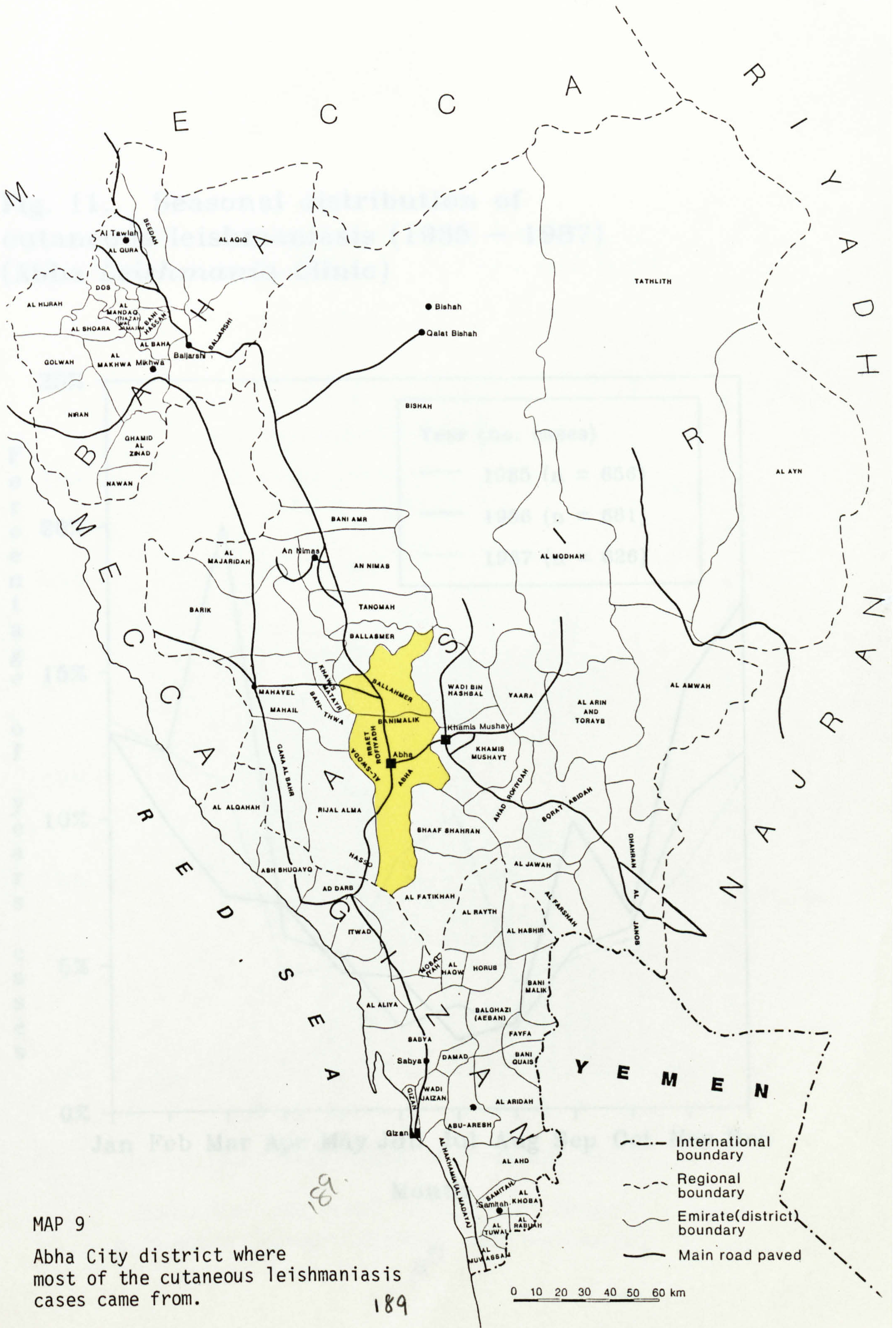
1.2 Monthly distribution of the disease

The monthly distribution of patients seen in the Abha *Leishmania* clinic is shown in Fig 11. The number of patients started to increase from October and reached the peak in December. This is a common feature over the three years' study. Other peaks in each individual years are seen in March, July and September 1985, 1987 and 1986 respectively.

The correspondence between the monthly distribution of patients and the monthly distribution of *Ph.sergenti* is shown in Figure 12.

1.3 Sex and age distribution

The sex and age distribution of the 626 patients seen during 1987 in the Abha *Leishmania* clinic are shown in Figures 13 and 14 respectively. The highest incidence was observed in the under 15 years age group with 54.8% of total cases. With regards to the sex distribution, 69% of the total cases were male.



MAP 9

Abha City district where most of the cutaneous leishmaniasis cases came from.

Fig. 11. Seasonal distribution of cutaneous leishmaniasis (1985 - 1987) (Abha *Leishmania* Clinic)

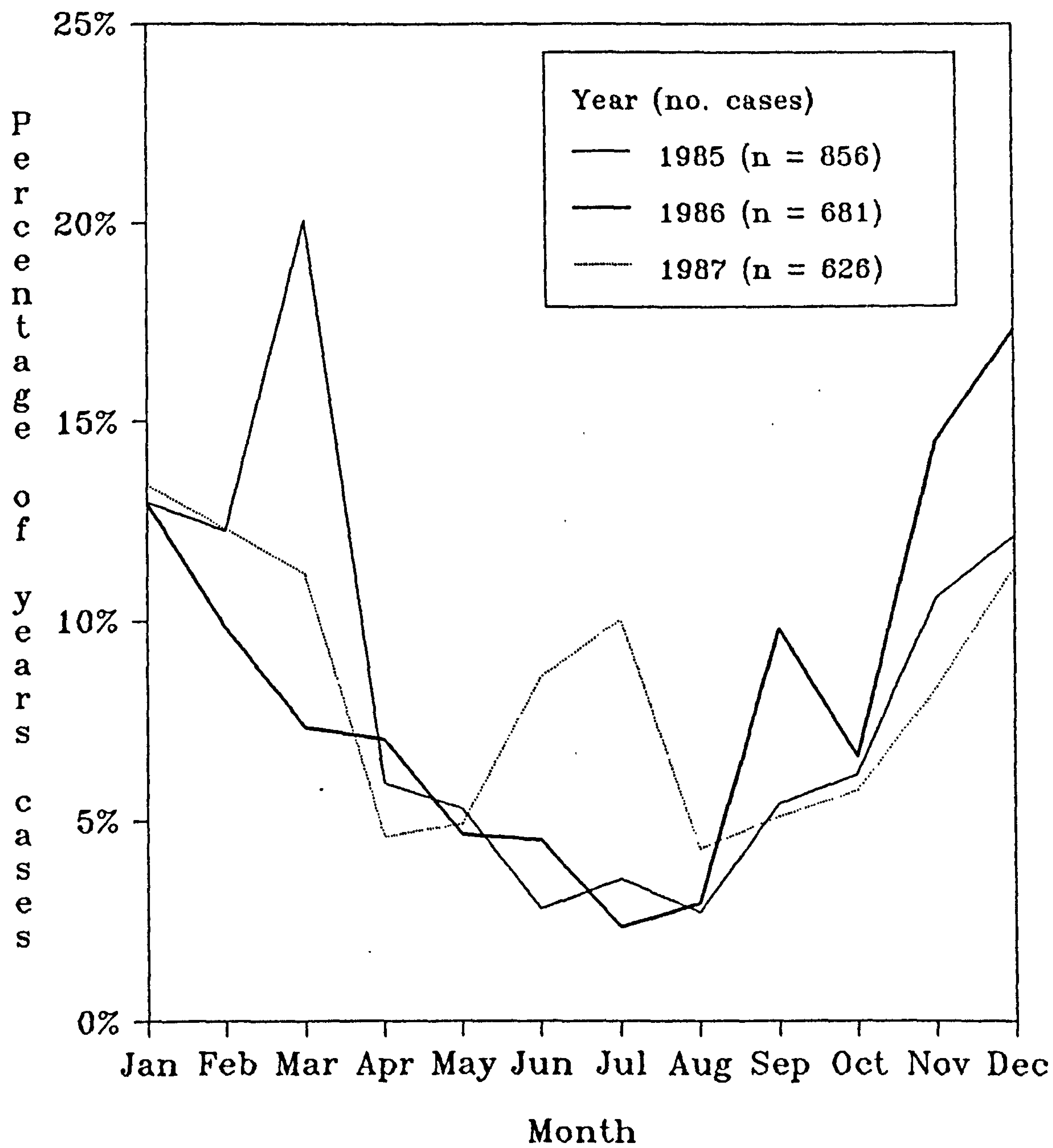


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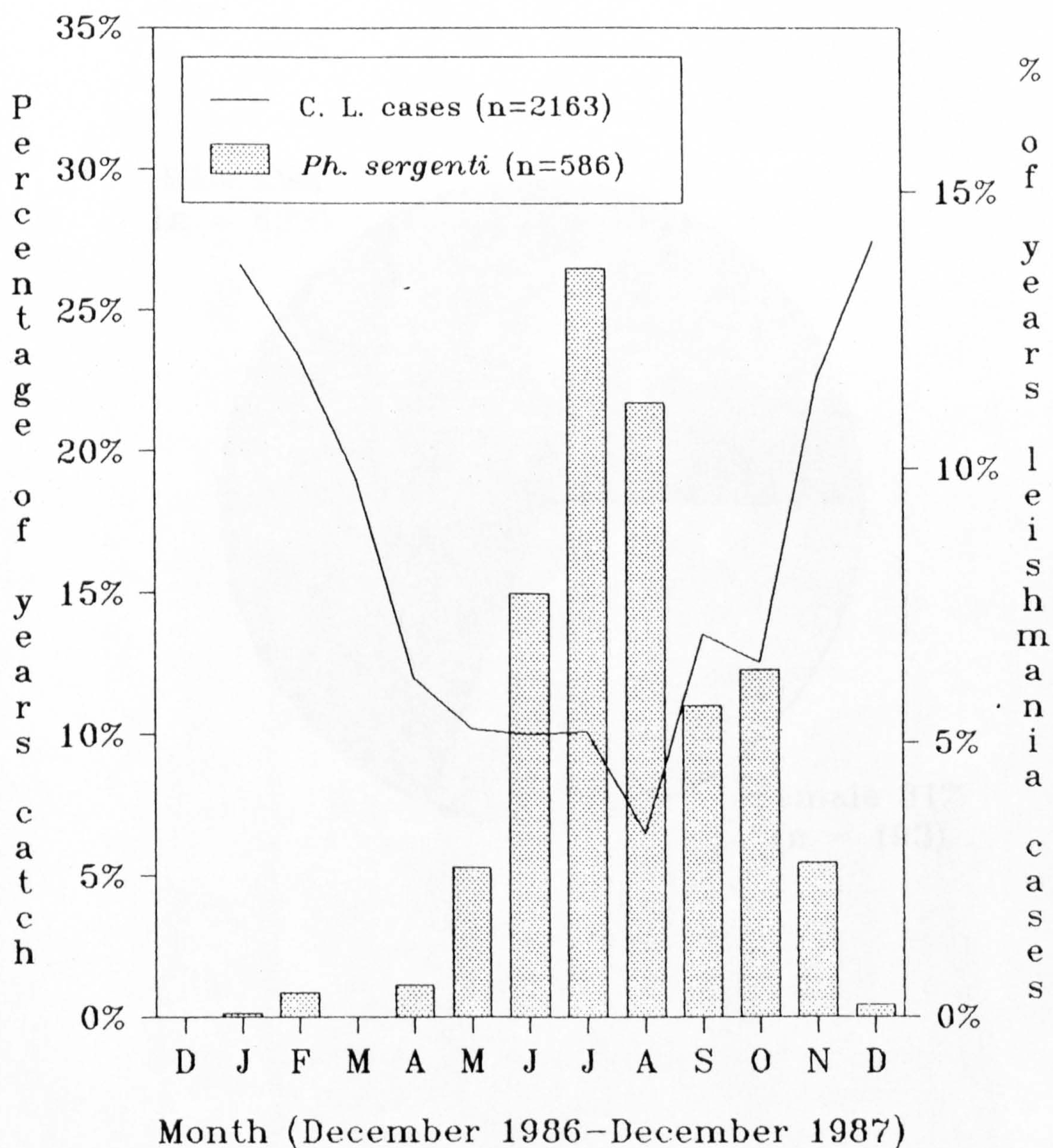
Fig. 12. Seasonal distribution of *Ph. sergenti* and cutaneous leishmaniasis (Highlands: Asir Province, sexes combined)



Sandflies collected using light traps and sticky traps (indoors and outdoors)

191

Fig. 13. Sex of cutaneous leishmaniasis patients (Abha *Leishmania* Clinic, 1987)

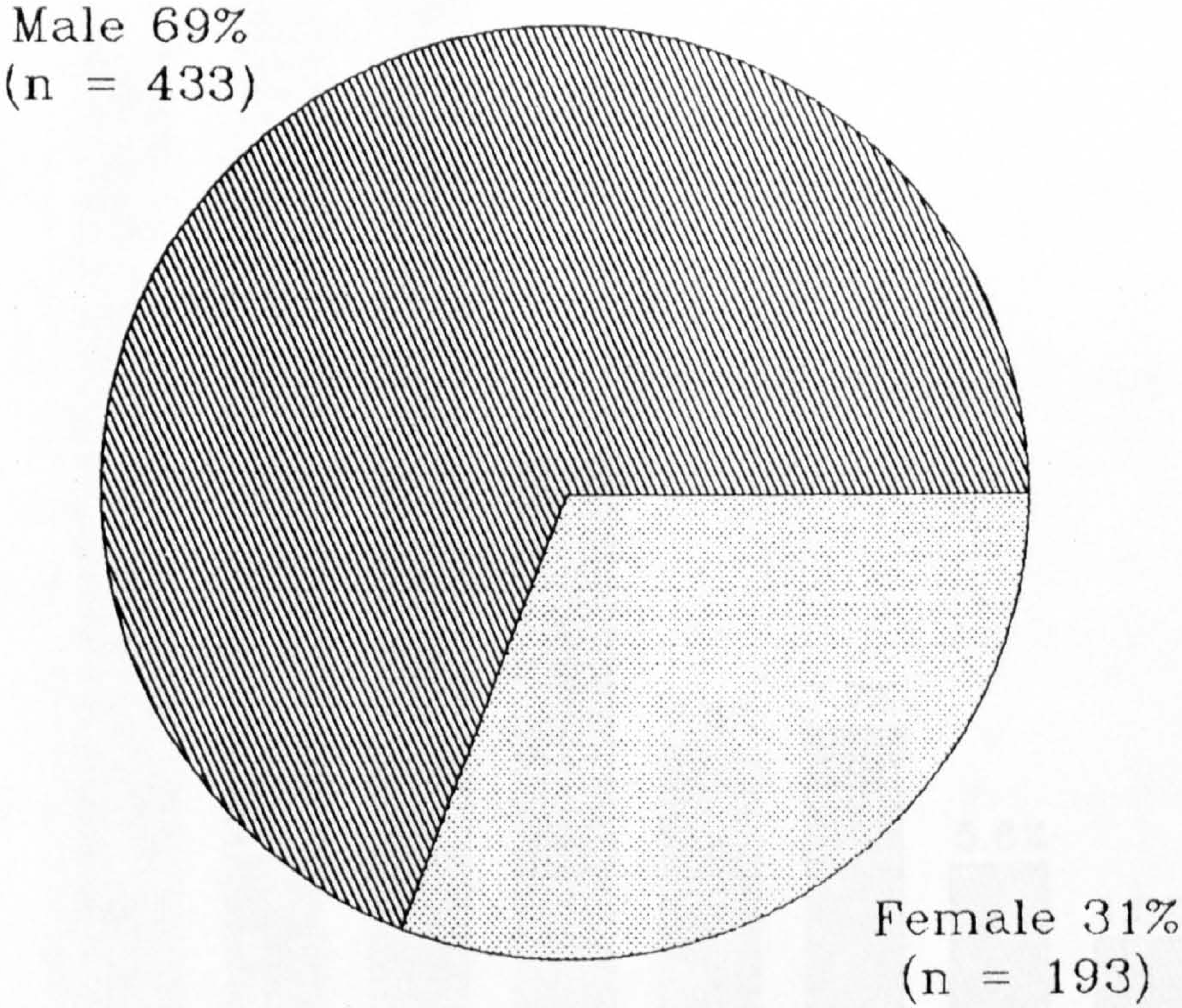
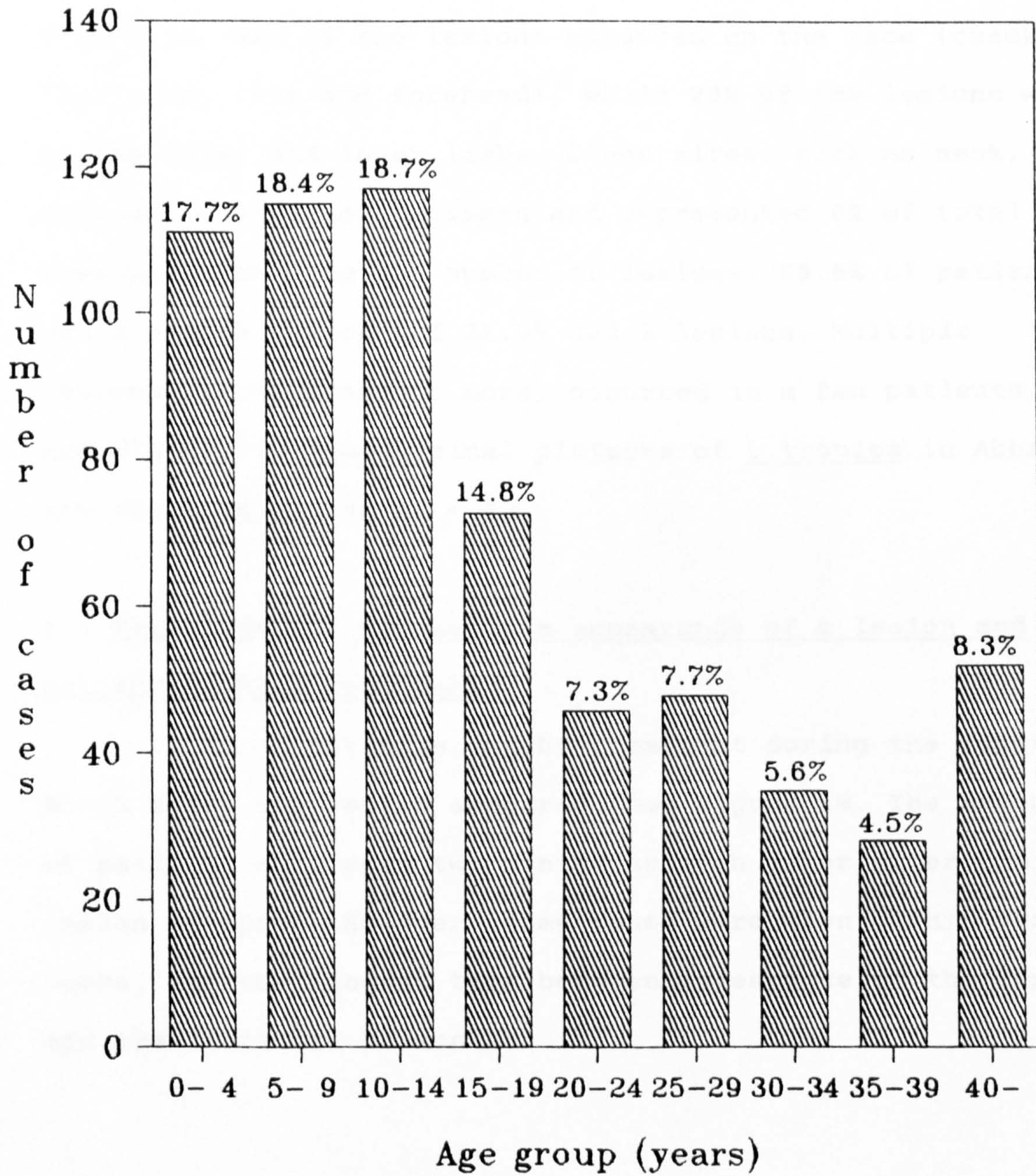


Fig. 14. Age distribution of cases of cutaneous leishmaniasis (Abha *Leishmania* clinic, 1987)



1.4 Nationality

As is shown in Figure 15, 79% of the patients were Saudi and 21% non-Saudi.

1.5 Number and site of lesions

The anatomical distribution of lesions is shown in Figure 16, 69% of the lesions occurred on the face (cheek, lip, nose, chin and forehead), while 23% of the lesions were on the upper and lower limbs. Other sites, such as neck, ear and eyelid were not uncommon and represented 8% of total lesions. Regarding the number of lesions, 65.8% of patients had a single lesion and 22.5% had 2 lesions. Multiple lesions (three scars or more) occurred in a few patients, see Figure 17. The clinical pictures of *L.tropica* in Abha are shown in plates 18 - 22.

1.6 Time interval between the appearance of a lesion and patient seeking treatment

11.8% of patients sought treatment during the first month after the lesion appeared, see Figure 18. The majority of patients were seen two months or even later after their lesion appeared. However, some cases were seen within two weeks, and the longest time between appearance of the lesion and treatment was 24 months.

Fig. 15. Nationality of cutaneous leishmaniasis patients (Abha *Leishmania* Clinic, 1987)

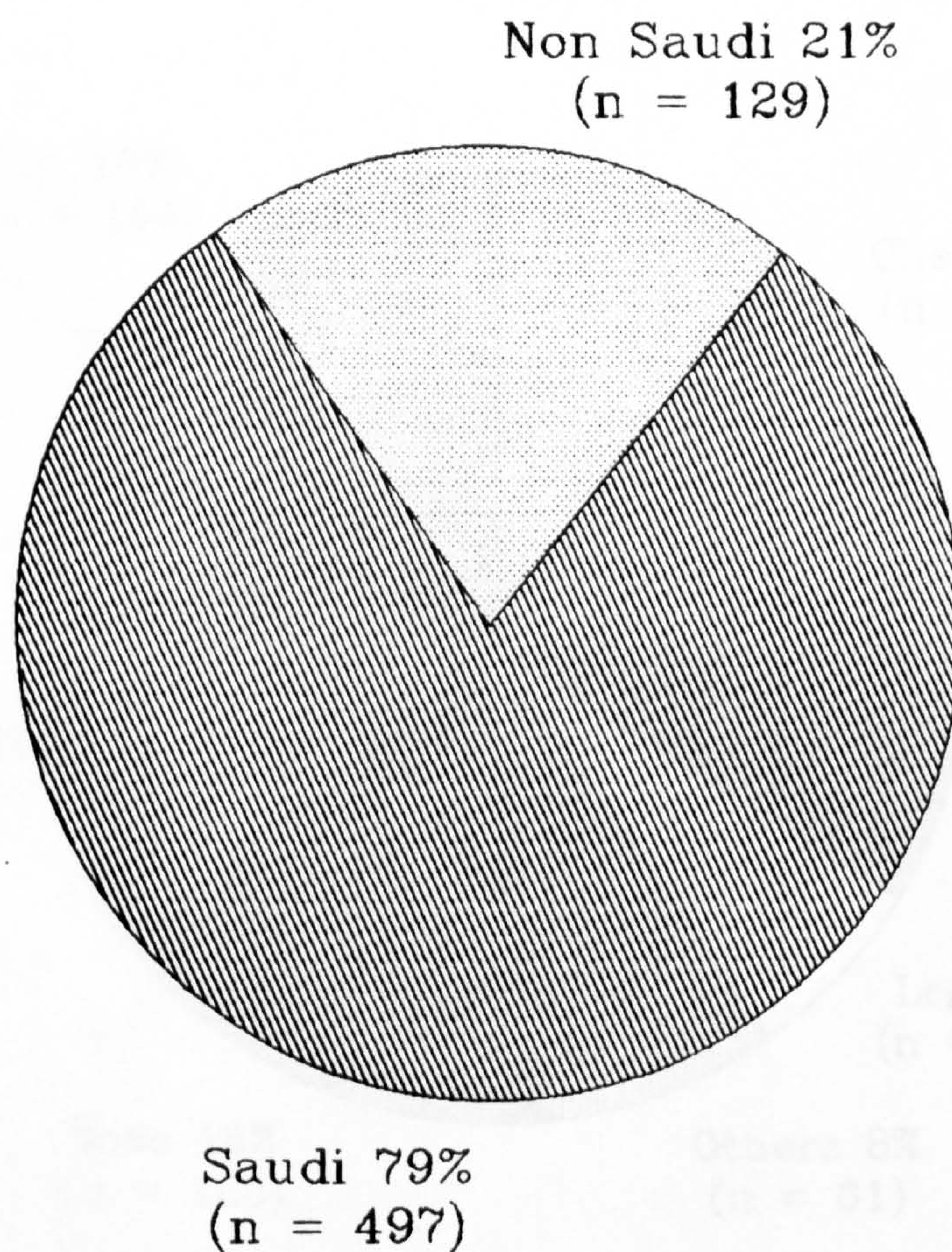


Fig. 16. Site of lesions (Abha *Leishmania* Clinic, 772 lesions)

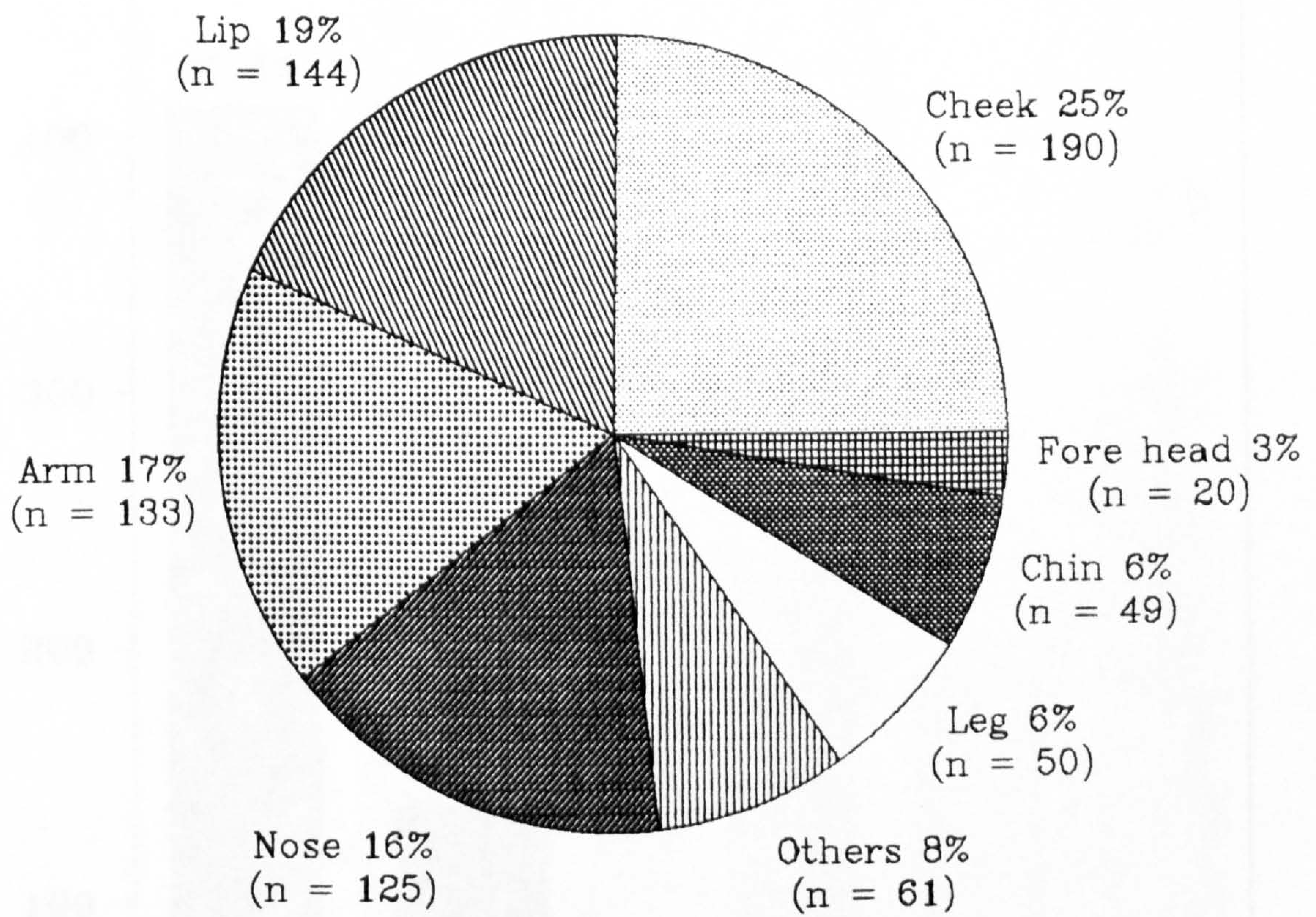


Fig. 17. Distribution of cutaneous leishmaniasis cases according to the number of lesions (Abha *Leishmania* Clinic, n = 626)

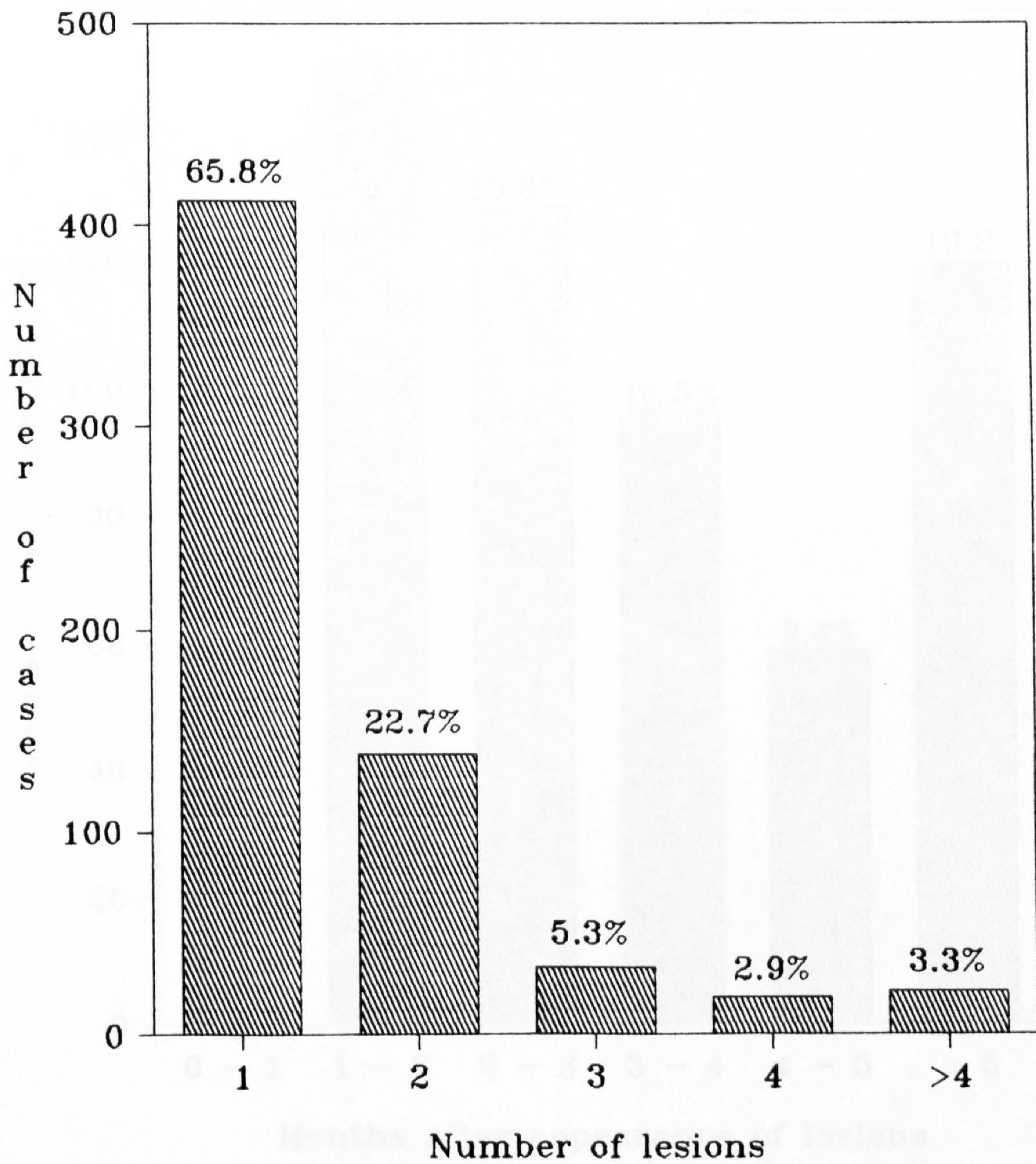
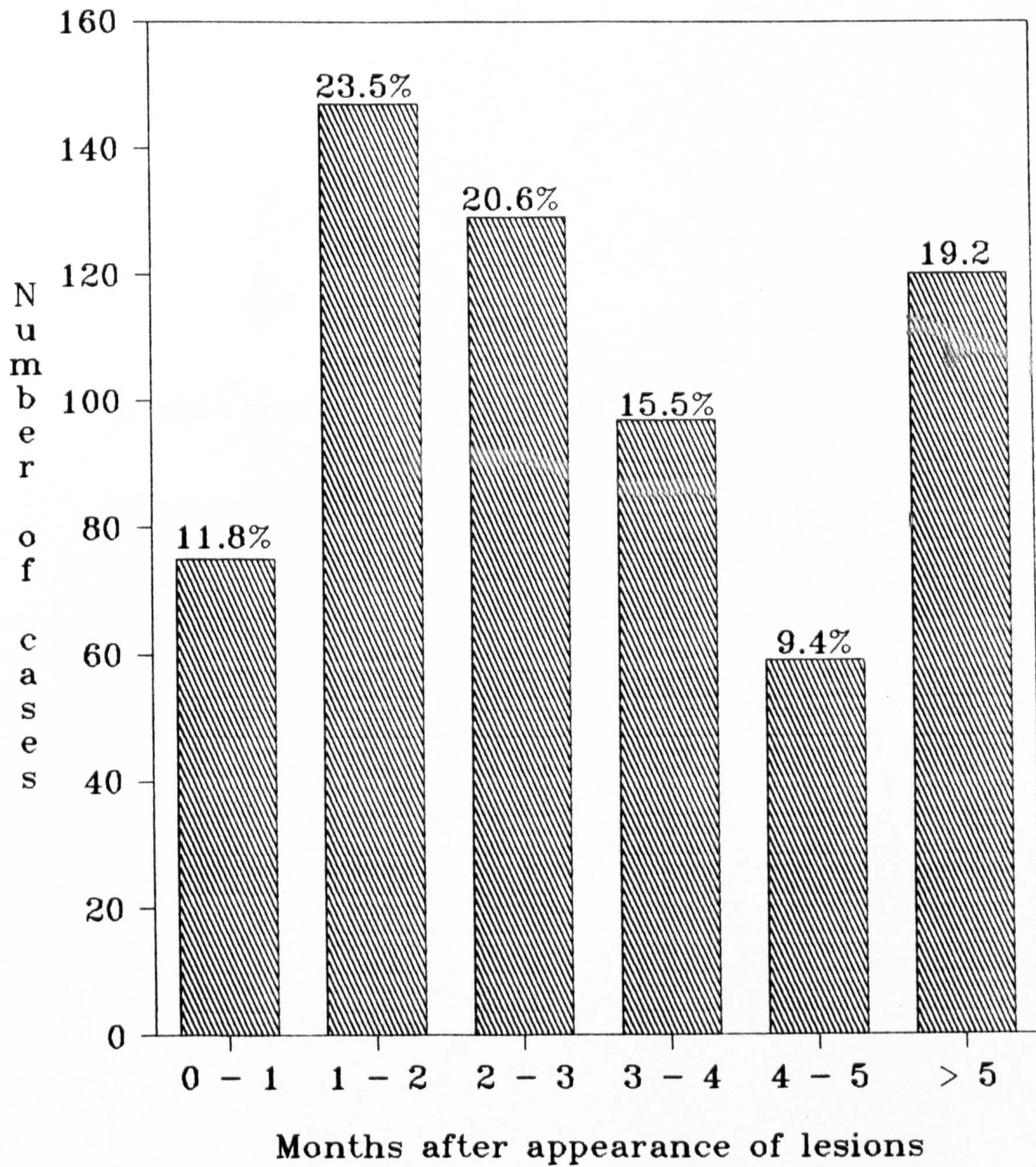


Fig. 18. The time interval between the appearance of lesions and seeking tratment (n = 626)

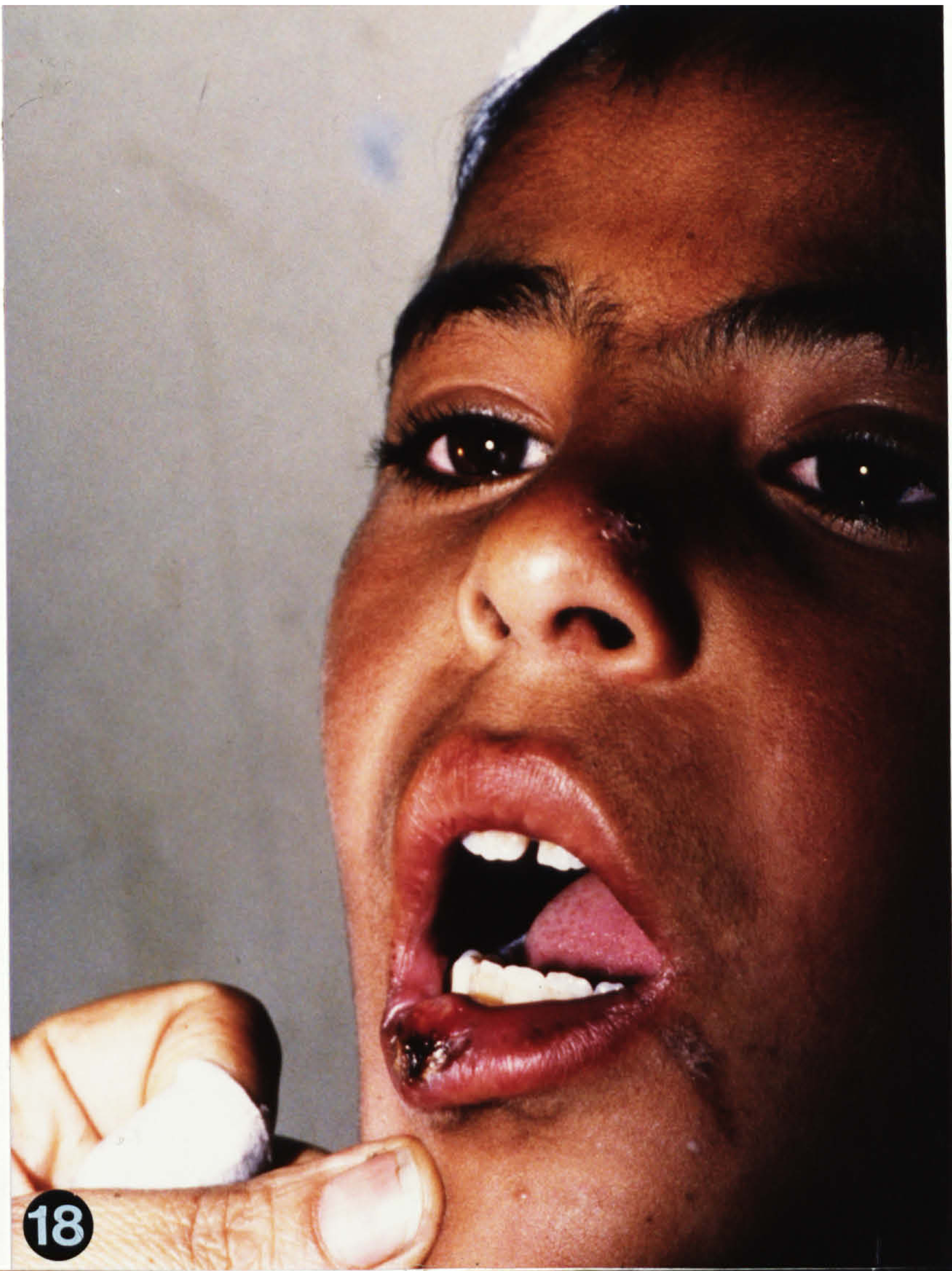


Clinical pictures of *L.tropica* lesions

Plate 18. Early lesions on nose, lip and chin.

Plate 19. Crusted, chronic lesions on nose and face in an 8 year-old boy.

Plate 20. Early lesion on nose of a young girl.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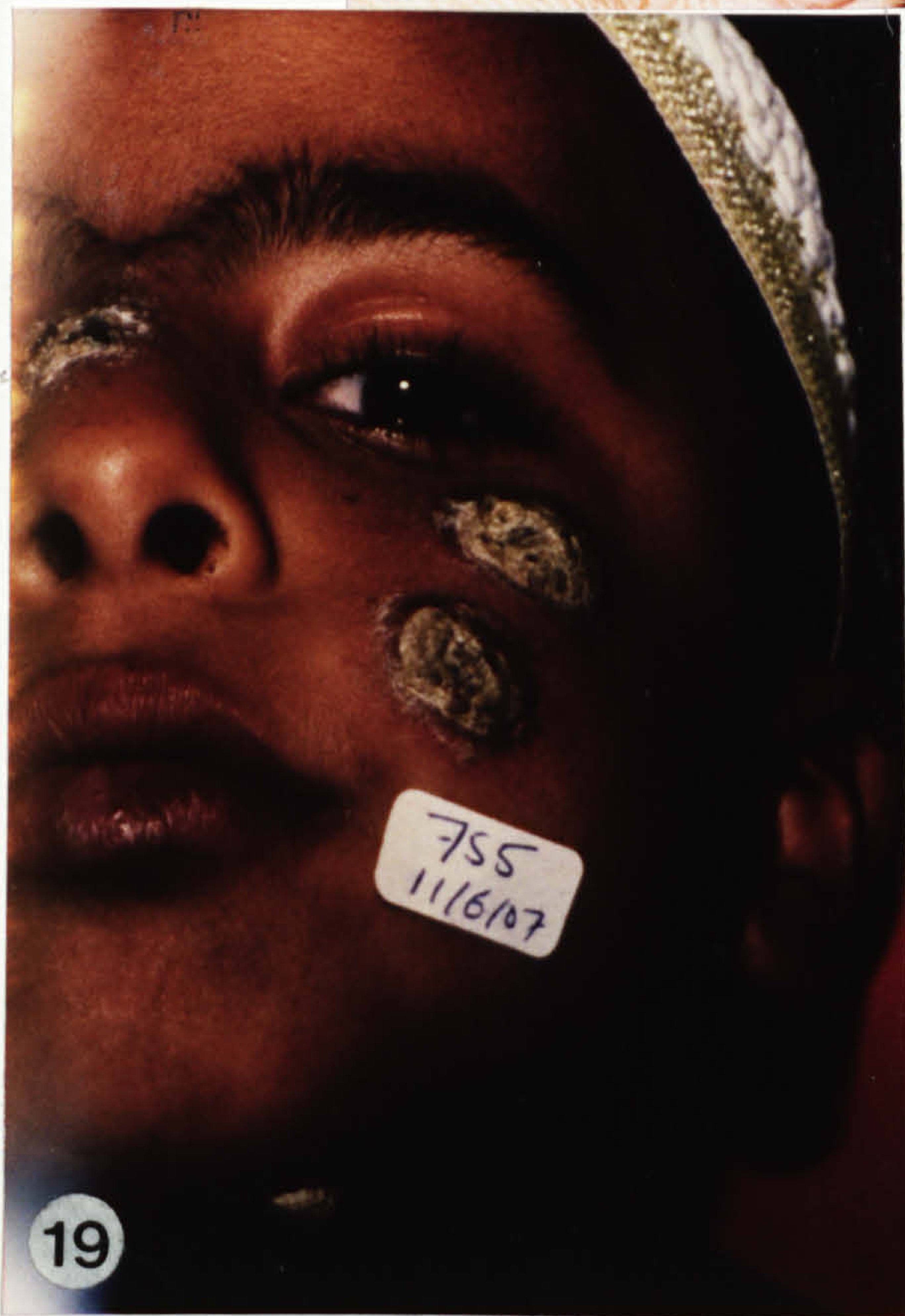
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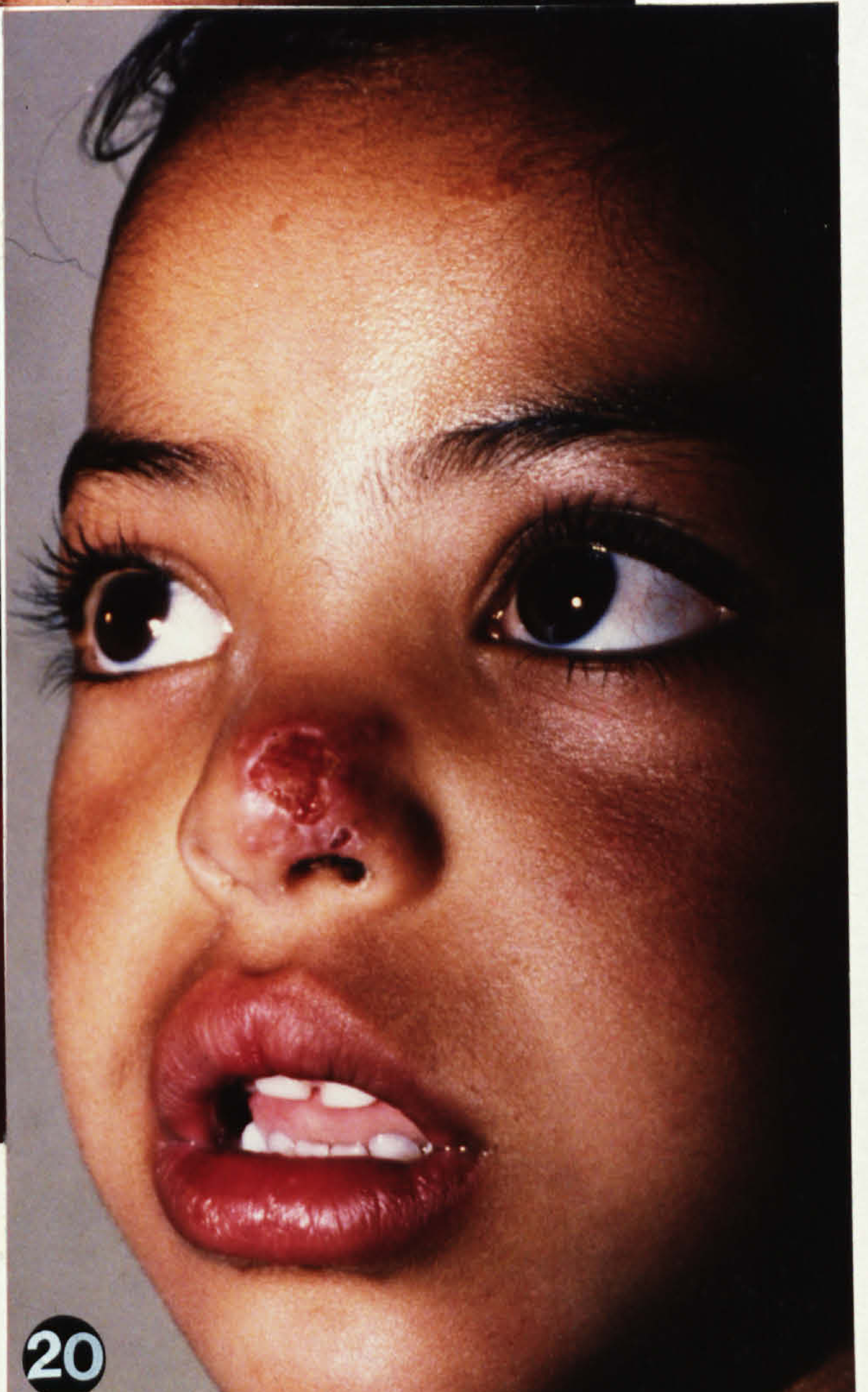
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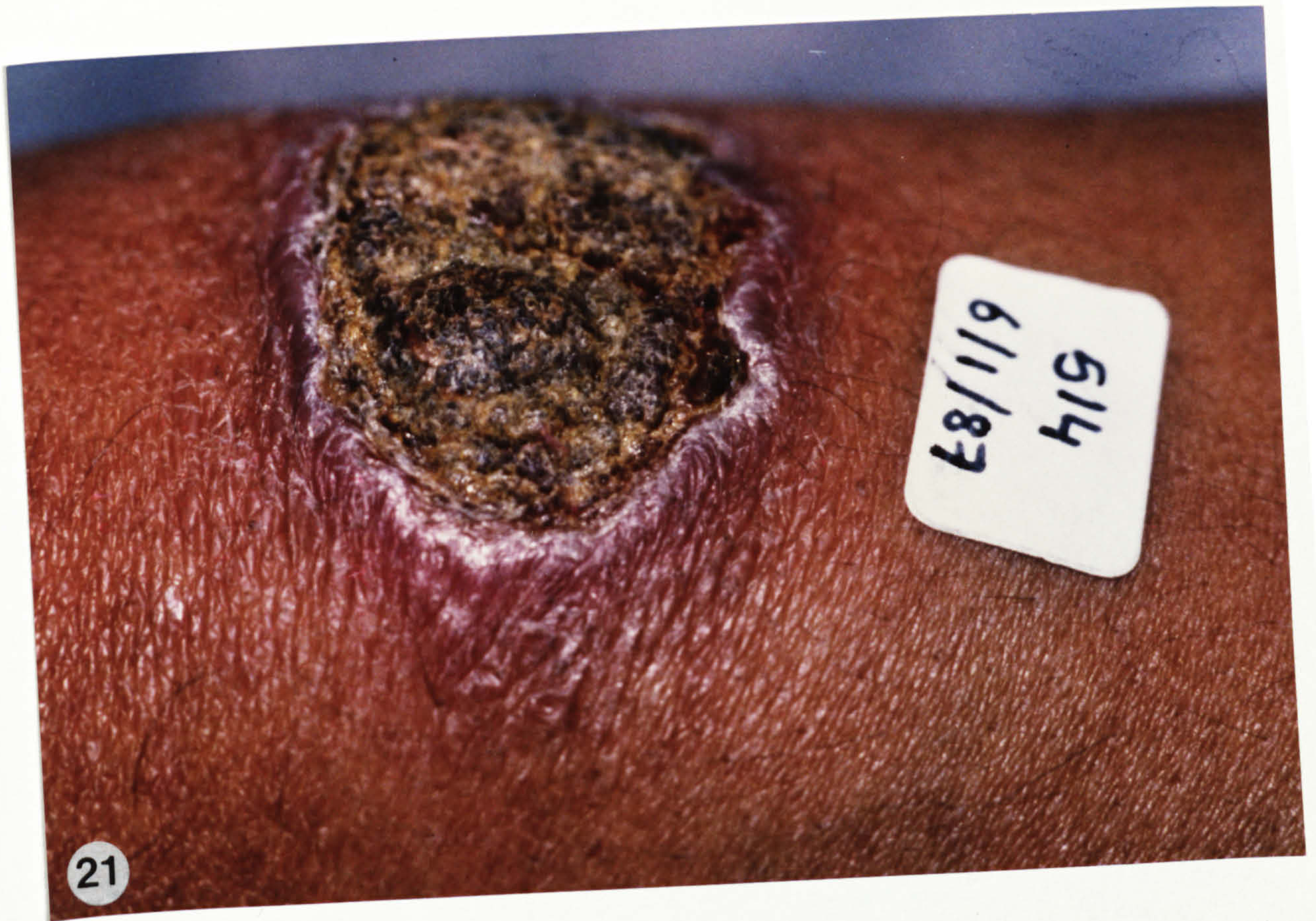
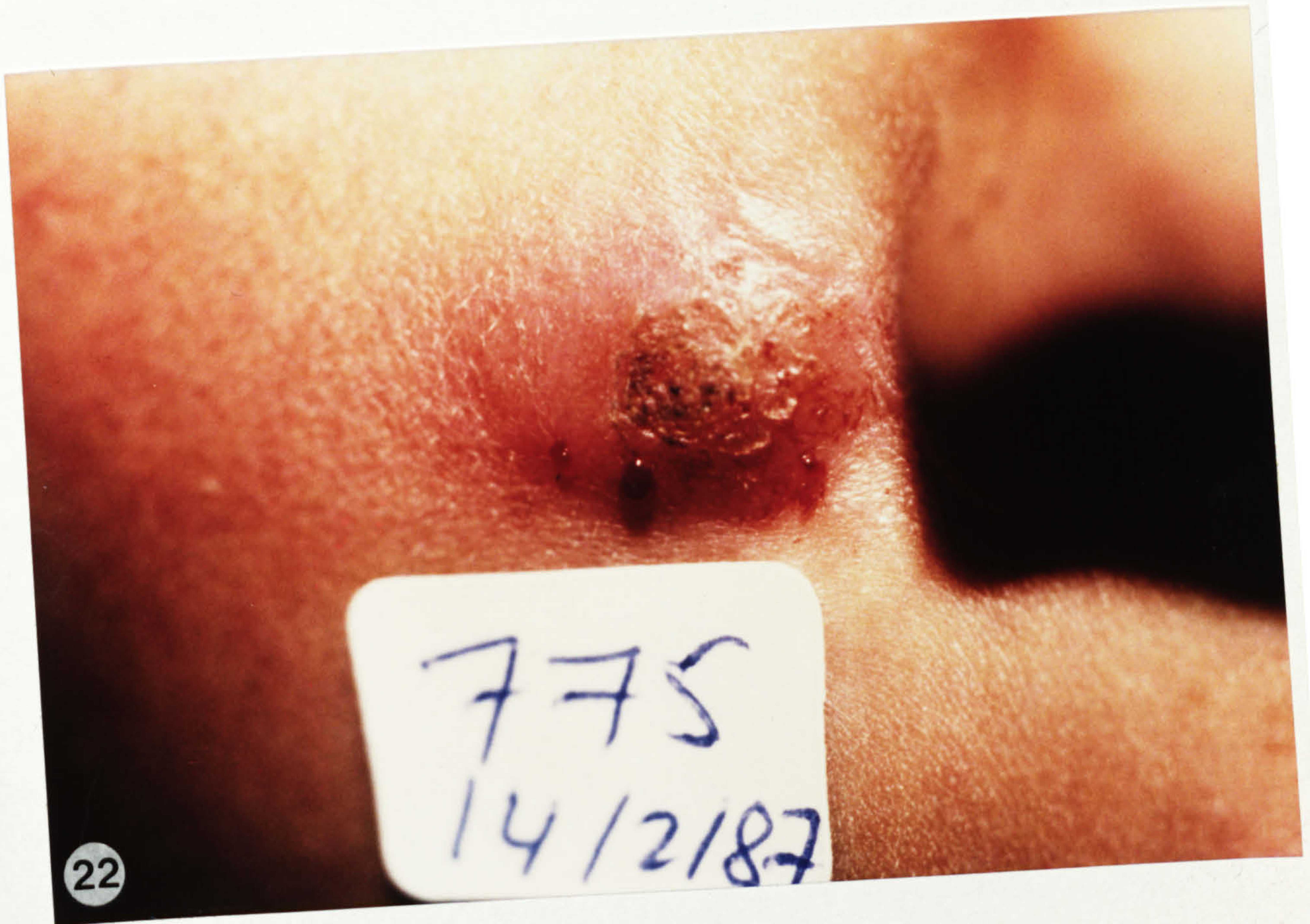
Clinical pictures of *L.tropica* lesions (contd.)

Plate 21. Chronic lesion on arm of an adult male which resembles *L.major*.

Plate 22. Large indurated lesion on arm with early ulceration.





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2. Culture

2.1 Primary isolation in the Abha laboratory

By the end of the field work, 191 specimens from patients with positive smears of CL (see plate 23) were cultured, 141 collected from the Abha *Leishmania* clinic, and 50 from Al-Baha province.

All the primary isolates were made in Evans' modified Tobie's medium, with 0.2 ml PBSS overlay. This proved to be a good medium for primary isolation. The subcultures were made in either NNN medium or Evans' modified Tobie's medium, except for samples to be transferred which were made in the special transport medium mentioned in Chapter III, 3.1.2.

The positivity rate of primary isolations was 45% (86 cultures out of 191 were positive). As mentioned previously in the methodology, Chapter III, 3.2.1) the specimens were obtained from the patients initially by dental broach, which was later replaced by skin snip biopsies or hypodermic needle aspirations. The positivity rates of these methods are shown in Table 23. The culture positivity rate was improved somewhat towards the mid-phase of the field work, a reflection of better laboratory techniques and expertise.

Positive cultures (Table 24 and Annex 2) were obtained from lesions with one week's duration, eg cultures No.A87 and A91. Isolates from lesion a year or more old were obtained also, eg cultures No. A40, A41 and B17. The isolates were collected from different localities and more

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than twenty localities were represented by one or more isolates. Details of positive cultures according to type of lesions, site and duration are shown in Annex 2.

The majority of positive cultures became positive within three to four days after inoculation. Nevertheless some cultures were positive after 2 days and a few, such as culture No.A68, was positive 28 days after the culture date. However, the mean of the interval between culture and day of positivity calculated from 40 positive cultures was 4.6 days, SD = 3.7 days.

The period of culture positivity of primary isolates varied from one culture to another. From the laboratory experience in Abha, we found that cultures continued to be positive without subculture for up to 3-4 months, eg, cultures, No.A73, A96 and 106. Nevertheless, some cultures which had been positive for a week or two became negative. The culture inoculation was carried out using aseptic technique and, with the exception of a few accidents at the start of the study, all cultures were clean and not contaminated. In the contaminated cultures, gentamycin (50 ug/ml) were added and, after one or two subcultures, they became clean and free of bacteria. Cultures with fungal contamination were rare.

2.2 Subcultures transported to the LSHTM

All the positive cultures which had been obtained in primary isolation in our laboratory in Abha were subcultured

TABLE 23 Primary isolations from cutaneous leishmaniasis patients: culture results according to the technique used for culture inoculation.

Inoculation technique	Number of specimens	Number of positive cultures	Percentage positive
Dental broach	24	0	0%
Biopsy by skin punch and scalpel	53	28	52.8%
Hypodermic needle aspiration	114	58	50.9%
Total:	191	86	45%

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TABLE 24 Results of isoenzyme typing of cutaneous leishmaniasis isolates from southwest Saudi Arabia and the geographic distribution of zymodemes (information on the patients' age, nationality, and the site and duration of lesions are in annex 2).

Isolate designation	Species	Zymodeme	Emirate (district)	Village	Note
MHOM/SA/87/A25	L. tropica	LON-72	Abha	Abha	H
MHOM/SA/87/A26	L. tropica	LON-72	Abha	Shaar	H
MHOM/SA/87/A29	L. tropica	LON-73	Abha	Bani-Malik	H
MHOM/SA/87/A32	L. tropica	LON-72	Bellahmar	Al-Mawaen	H
MHOM/SA/87/A36	L. tropica	LON-72	Mahayel	Mahayel	L
MHOM/SA/87/A42	L. tropica	LON-72	Abha	Abha	H
MHOM/SA/87/A51	L. tropica	LON-72	Abha	Abha	H
MHOM/SA/87/A53	L. tropica	LON-72	Abha	Abha	H
MHOM/SA/87/A54	L. tropica	LON-72	Bellahmar	Abel	H
MHOM/SA/87/A59	L. major	LON-4	Tath-Lith	Tath-Lith	D
MHOM/SA/87/A60	L. tropica	LON-72	Abha	Abha	H
MHOM/SA/87/A63	L. tropica	LON-72	Abha	Abha	H
MHOM/SA/87/A65	L. tropica	LON-72	Khamis-mushayt	Kamis-mushayt	H
MHOM/SA/87/A67	L. tropica	LON-72	Bellahmar	Abel	H
MHOM/SA/87/A68	L. tropica	LON-72	Abha	Bani-Rezam	H
MHOM/SA/87/A69*	L. major	LON-1	Abha	Abha	H
MHOM/SA/87/A73	L. tropica	LON-72	Bellahmar	Abel	H
MHOM/SA/87/A76	L. tropica	LON-72	Abha	Abha	H
MHOM/SA/87/A77	L. tropica	LON-72	Sorat-Abidah	Sorat-Abidah	H
MHOM/SA/87/A78	L. tropica	LON-72	Abha	Bani-Malik	H
MHOM/SA/87/A87	L. tropica	LON-72	Abha	Abha	H
MHOM/SA/87/A96	L. tropica	LON-72	Abha	Abha	H

Notes:
H-Highlands, L-Lowlands, D-Desert area to the east of the Asir mountains.

*A Sudanese patient

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TABLE 24 (Continued) Results of isoenzyme typing of cutaneous leishmaniasis isolates from southwest Saudi Arabia and the geographic distribution of zymodemes (information on the patients' age, nationality, and the site and duration of lesions are in annex 2).

Isolate designation	Species	Zymodeme	Emirate (district)	Village	Note
MHOM/SA/87/A106	L. tropica	LON-72	Abha	Abha	H
MHOM/SA/87/A113	L. tropica	LON-72	Abha	Rabeat-Rufadh	H
MHOM/SA/87/A114	L. tropica	LON-72	Abha	Abha	H
MHOM/SA/87/A115	L. tropica	LON-72	Al-Namis	Bani-Omar	H
MHOM/SA/87/A117	L. tropica	LON-72	Abha	Abha	H
MHOM/SA/87/A122	L. tropica	LON-63	Mahayel	Mahayel	L
MHOM/SA/87/A126	L. tropica	LON-71	Sorat-Abidah	Sorat-Abidah	H
MHOM/SA/87/A127	L. tropica	LON-71	Abha	Bani-Malik	H
MHOM/SA/87/A129	L. tropica	LON-63	Gizan	Bal-Ghazi	L
MHOM/SA/87/A130	L. tropica	LON-63	Rijal-Alma	Rijal-Alma	L
MHOM/SA/87/A137	L. tropica	LON-10	Abha	Abha	H
MHOM/SA/87/B1	L. tropica	LON-72	Bal-Jarshi	Bal-Jarshi	H
MHOM/SA/87/B3	L. tropica	LON-72	Dos	Dos-Bani-Ali	H
MHOM/SA/87/B11	L. tropica	LON-72	Al-atawlah	Al-atawlah	H
MHOM/SA/87/B13	L. tropica	LON-72	Bani-Hassan	Wadi-Sadar	H
MHOM/SA/87/B15	L. tropica	LON-72	Dos	Dos-Al-Namah	H
MHOM/SA/87/B16	L. tropica	LON-72	Al-atawlah	Al-atawlah	H
MHOM/SA/87/B17	L. tropica	LON-72	Baha	Al-Mousa	H
MHOM/SA/87/B503	L. tropica	LON-72	Baha	-	-
MHOM/SA/87/B635	L. tropica	LON-10	Bal Jarshi	Bani-Kaber (Al-Hadab)	H
MHOM/SA/87/B812	L. tropica	LON-72	Baha	-	-
MHOM/SA/87/B848	L. tropica	LON-72	Baha	-	-

Notes:
H-Highlands, L-Lowlands, D-Desert area to the east of the Asir mountains.

L.tropica in smear

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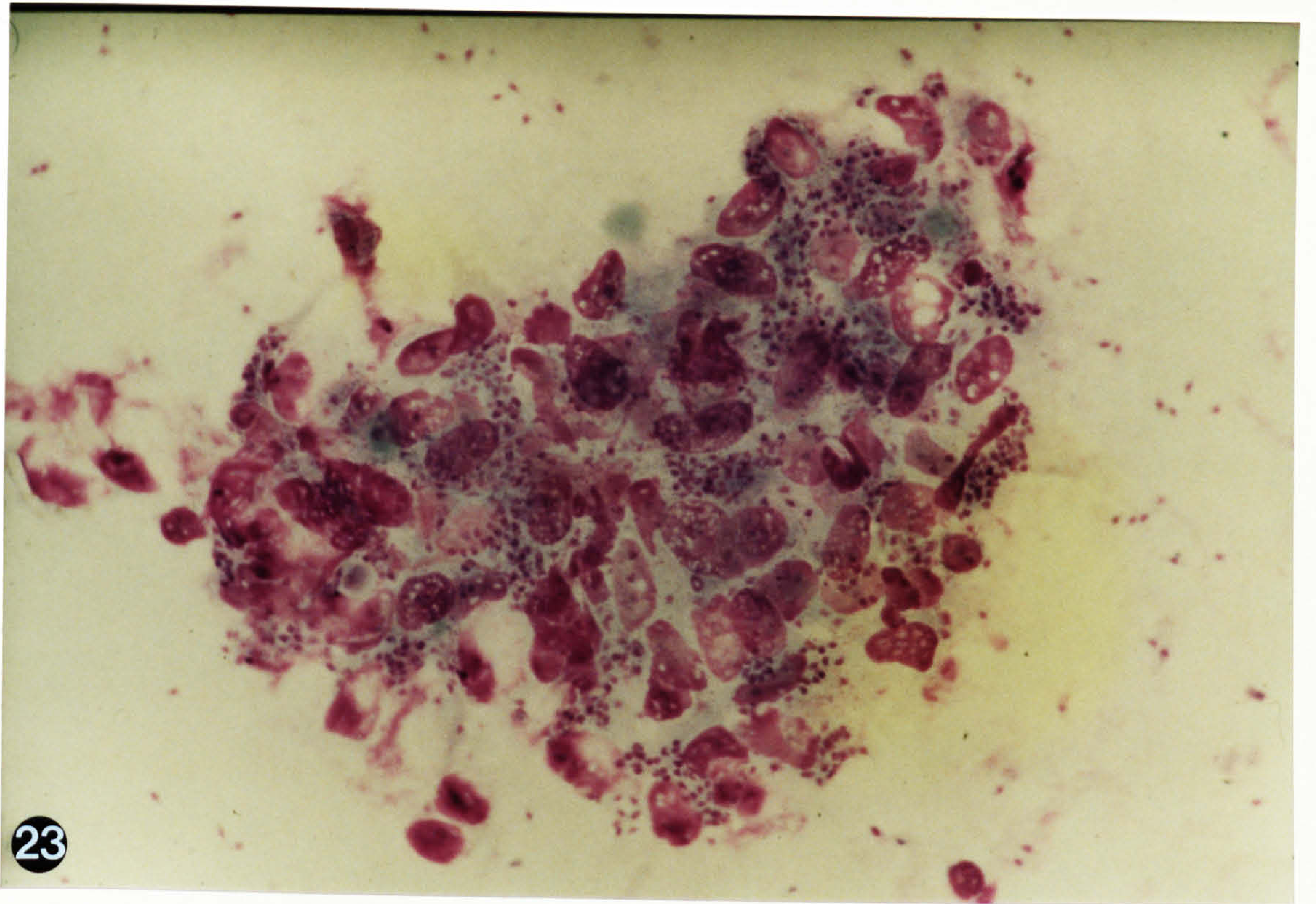
Plate 23. Heavily infected macrophages in a skin smear from a patient with cutaneous leishmaniasis due to *L.tropica* (Abha)


23

in transport medium and sent to London. Fifty out of 86 subcultures grew well and were preserved in the cryobank, as well as being grown in bulk and beaded for later isoenzyme studies.

2.3 Isoenzyme typing

2.3.1 Species and zymodemes

Forty four isolates from cutaneous leishmaniasis patients from Asir and Al-Baha were tested, using up to 12 enzymes. Under the enzyme running condition (Tables 11 and 12), the enzyme resolutions were optimal and there were no difficulties in allocating the isolates to an individual taxon and/or zymodeme. Apart from one isolate from a Saudi patient and another from a Sudani patient which proved to be *L.major*, the rest of the isolates were *L.tropica*. Five different zymodemes (LON-zymodemes) were found among the *L.tropica* isolates. These were:-

(i) Zymodeme LON-10 in two isolates (MHOM/SA/87/B635) from Al-Baha province, Bal Jarshi Emirate, the second one (MHOM/SA/87/A137) from Abha city approximately 300 km away from the first one. It should be mentioned that the same zymodeme was also seen in a sandfly isolate (ISER/SA/87/SSP 286) which was caught in Asir province from a village (Shuhat) 20 km northwest of Abha city.

(ii) Zymodeme LON-71: This zymodeme was seen in two isolates (MHOM/SA/87/126), (MHOM/SA/87/A127) and another

sandfly (ISER/SA/87/SSP454).

(iii) Zymodeme LON-63: in three isolates (MHOM/SA/87/A129), (MHOM/SA/87/A130) amd (MHOM/SA/87/A137).

(iv) Zymodeme LON-72: This zymodeme was the dominant one and was seen in the rest of the isolates (see Table 24)

(v) Zymodeme LON-73: Seen in one isolate (MHOM/SA/87/A29).

While two zymodemes (LON-10) and (LON-63) were reported previously by Le Blancq and Peters (1986b) and Peters et al.(1985), the other zymodemes (LON-71, LON-72 and LON-73)are reported here for the first time. Of the 12 enzymes examined LON 71 differed from LON-10 in one enzyme, 6PGD (see plates 24, 25, 26). LON-72 and LON-73 resembled LON-63 except two enzymes, PGM and SOD, (see plates 27, 29, 30) and three enzymes, PGM, MPI, SOD respectively (See plate 28). All the zymodemes of L.tropica in southwest Saudi Arabia are illustrated diagrammatically in Figure 19.
The zymodemes of two L.major isolates were LON-4 and LON-1. The isolates were from a Saudi patient and a Sudani patient respectively (see plates 31, 32).

2.3.2 Geographical distribution of zymodemes

As pointed out in section 2.2, the positive primary isolates were collected from different localities and more than 20 localities were represented. Map 10 shows the distribution of the zymodemes which were obtained from each Emirate (district). The village or tribal names are shown in Table 24. It is clear that zymodeme LON-72 is the

dominant zymodeme. Other zymodemes are represented by only one or two isolates. In addition to that, the following observations should be pointed out:-

(i) A striking observation was that zymodeme LON-63 was limited to the foothill areas of the lowlands, altitude 600-1100 m above sea level.

(ii) Three different zymodemes, LON-71, LON-72 and LON-73, were obtained from one locality, 10 km to the north of Abha City, (Bani-malik).

(iii) The L.major zymodeme, LON-4 which was found in the Saudi patient was from the Tethlith area which is an oasis at the junction of the Asir mountain plateau with the "Empty quarter"desert (see map 10). This zymodeme is the same as that found in many isolates from patients in the Eastern province of the country (Al-Hofuf area).

(iv) Zymodeme LON-1 which was identified from the Sudani patient revealed that the patient acquired his infection from the Sudan where this zymodeme exists.

2.4 Animal susceptibility

A total of 7 hamsters and 7 BALB/c mice were injected in the footpad with 7 isolates of L.tropica obtained from patients in the study area. The results are as follows:

(i) BALB/c mice

The mice injected with isolates nos. MHOM/SA/87/A46 and MHOM/SA/87/56 died after one month. No obvious thickness or lesion was observed and no amastigotes were seen in the direct smears. The rest of the mice were followed up weekly

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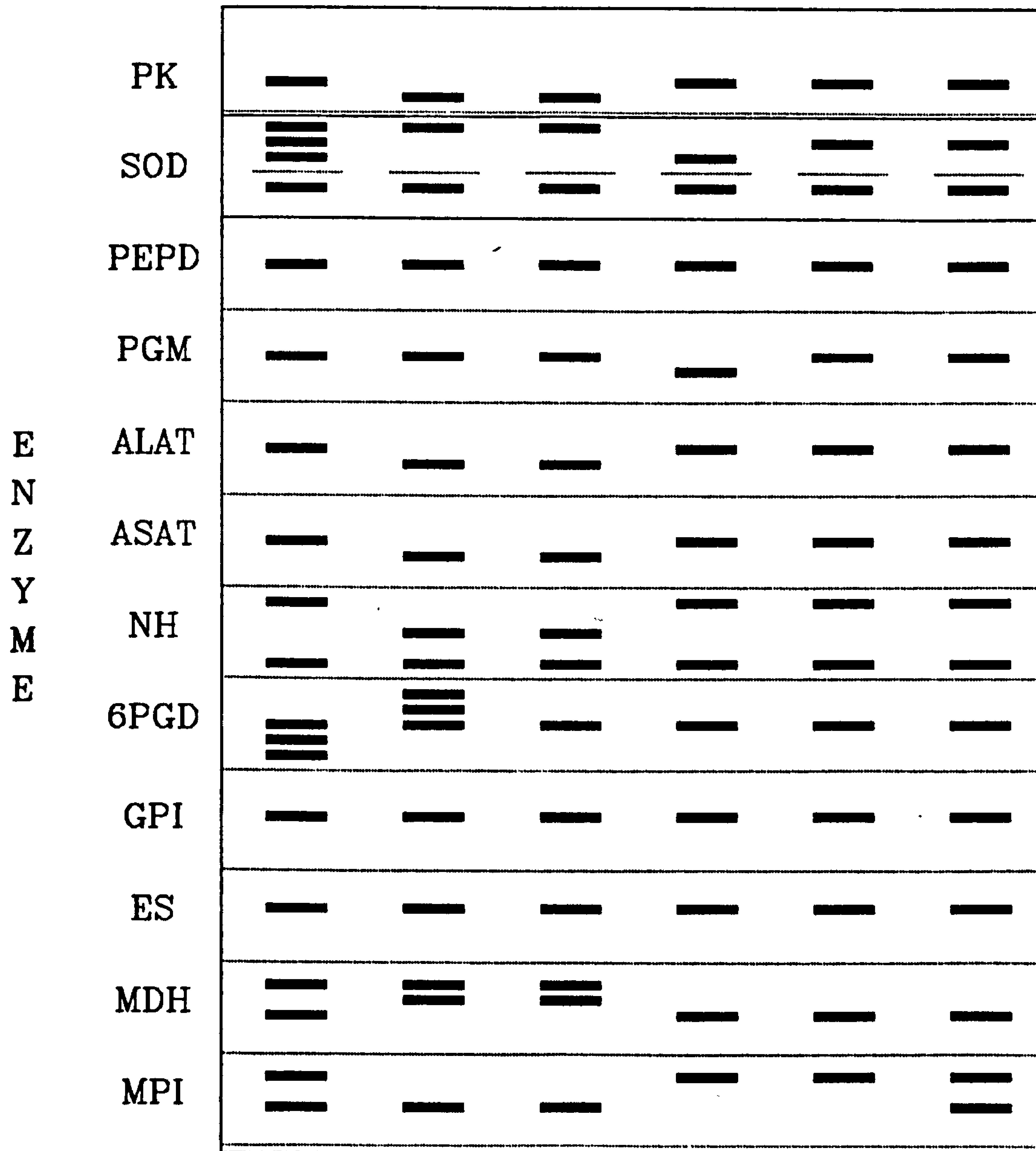
and the measurements showed no increase in footpad size beyond that of day 0, or in comparison with the controls (non-inoculated, other footpad). After 6 months' follow-up, all the mice were killed by anaesthesia and dissected, smears and cultures being from the footpad, spleen and liver, all of which were negative.

(ii) Hamsters

The footpads of three hamsters injected with isolates MHOM/SA/87/A113, MHOM/SA/87/A117 and MHOM/SA/87/A119 increased from 2.8 mm, 2.7 and 2.6 on day 0 to 3.2 mm, 3.1 and 3.1 mm after 36, 46 and 45 days respectively. A week later, specifically on days 42, 53 and 52 respectively, the size of the injected footpads dropped nearly to the same size as day 0, and continued without any remarkable thickness or obvious lesions. On day 95 all the hamsters were killed by anaesthesia and dissected. Impression smears and cultures from the site of injection were made as well as from the liver and spleen. The cultures were negative and no parasites were seen in the smears.

During the weekly follow-up a lesion in the neck of a hamster inoculated with isolate no. MHOM/SA/87/A119 was noticed three weeks after injection, measuring 2 x 3 cm. Biopsy and smear were taken from this lesion; no parasites were seen in the direct smear and the culture was negative for Leishmania promastigote.

Fig. 19. The electrophoretic patterns of 12 enzymes from cutaneous *Leishmania* isolates from southwest Saudi Arabia



Zymodeme LON–	7	10	71	63	72	73
Isolate No.	Reference	SSP286	SSP454	A129	Others	A29
		B635	A126	A130		
		A137	A127	A122		

—— indicates the origin

MAP 10

Geograpical distribution of L.tropica & L.major zymodemes in southwest Saudi Arabia.
L.tropica zymodemes LON - 71 ▲
LON - 72 ▲ LON - 73 ◆
LON - 63 ● LON - 10 ★
L.major zymodemes LON - 4 ■

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The isoenzyme zymograms of human and sandfly isolates of <u>Leishmania</u>. (As the identification of new isolates was made on the basis of a number of enzymes, no identification is stated on these figures except for the markers. See text for final identifications).

Key to the isolates:

A & B = human cutaneous leishmaniasis isolates

SSP = sandfly isolates

D = dog isolates

VL = human visceral leishmaniasis isolates

* = markers

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Plate 24. 6-phosphogluconate dehydrogenase (6PGD)

Samples	Identifications
1. MHOM/SU/60/OD	L.tropica (LON-7)*
2. MHOM/IL/00/Singer	L.tropica (LON-10)*
3. ISER/SA/87/SSP286	
4. MHOM/SA/00/Stiehl	L.tropica (LON-22)*
5. MHOM/SA/83/Giz33	L.tropica (LON-63)*
6. MHOM/SU/73/5ASKH	L.major (LON-1)*
7. MHOM/SA/87/A127	
8. MHOM/SA/87/A129	

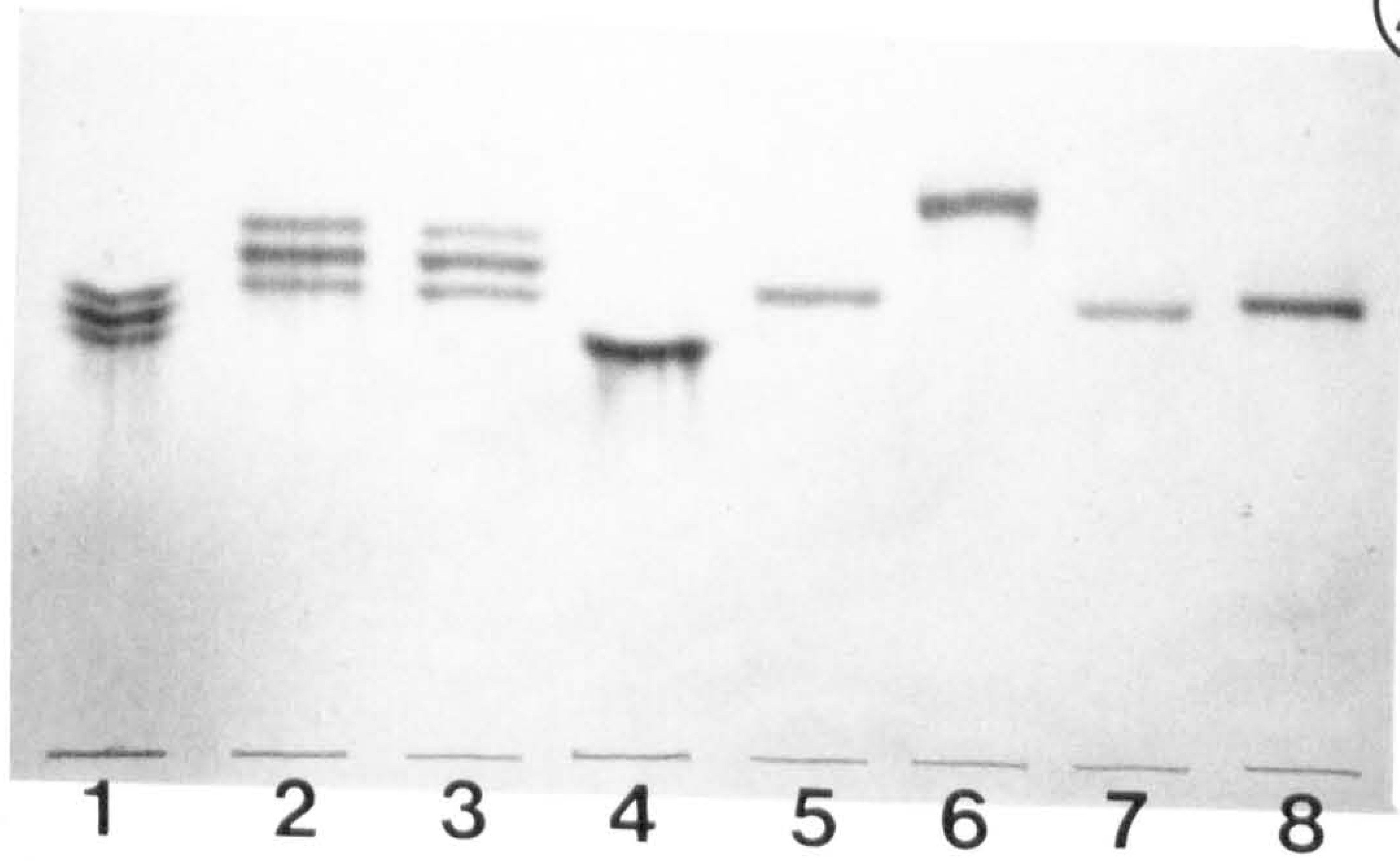
Plate 25. 6-phosphogluconate dehydrogenase (6PGD)

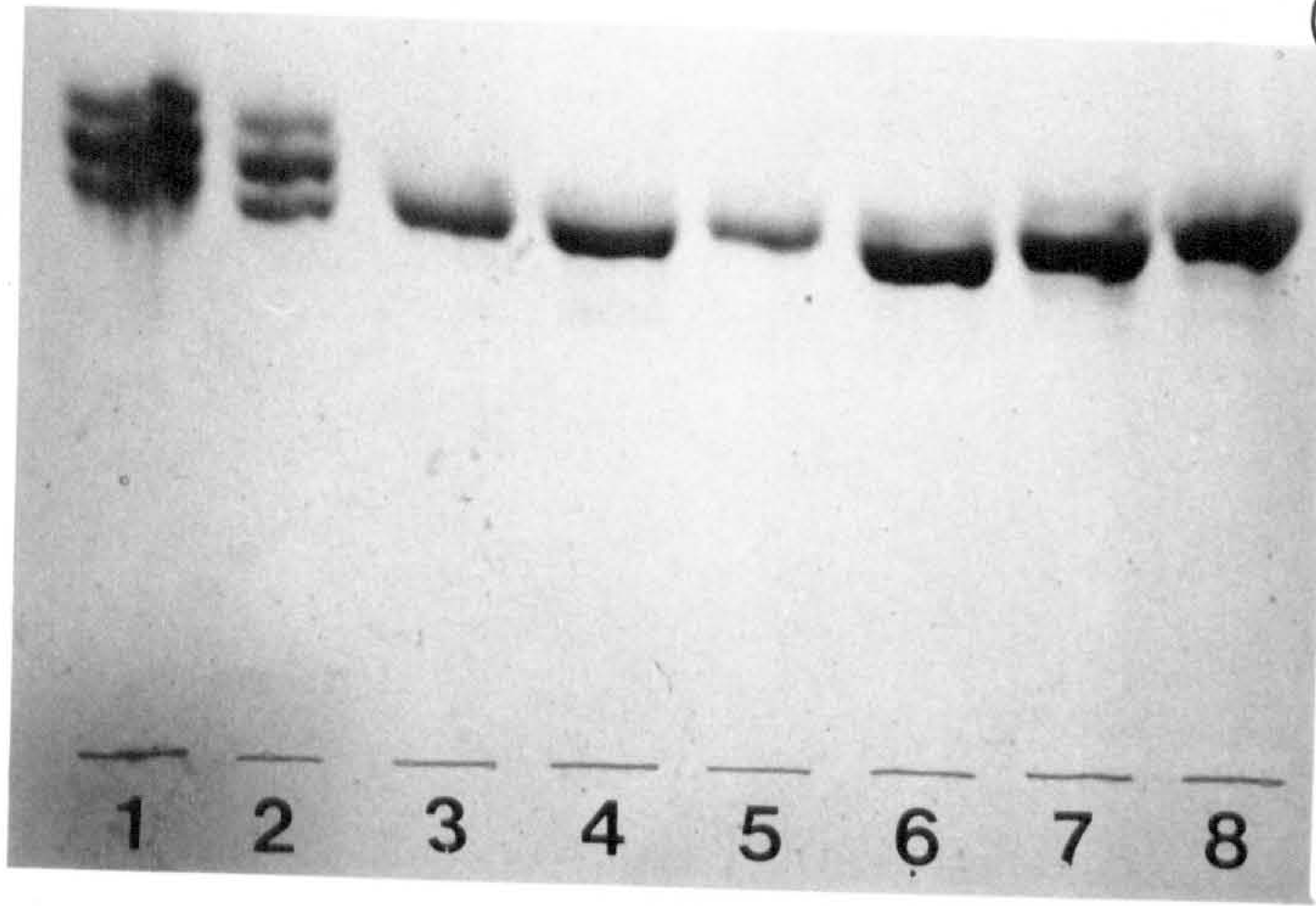
1. MHOM/IL/00/Singer	L.tropica (LON-10)*
2. ISER/SA/00/SSP286	
3. ISER/SA/87/SSP445	
4. MHOM/SA/87/A125	
5. MHOM/SA/87/A127	
6. MHOM/SU/73/32ASKH	L.tropica (LON-18)*
7. MHOM/SA/83/Giz33	L.tropica (LON-63)*
8. MHOM/SA/87/A130	

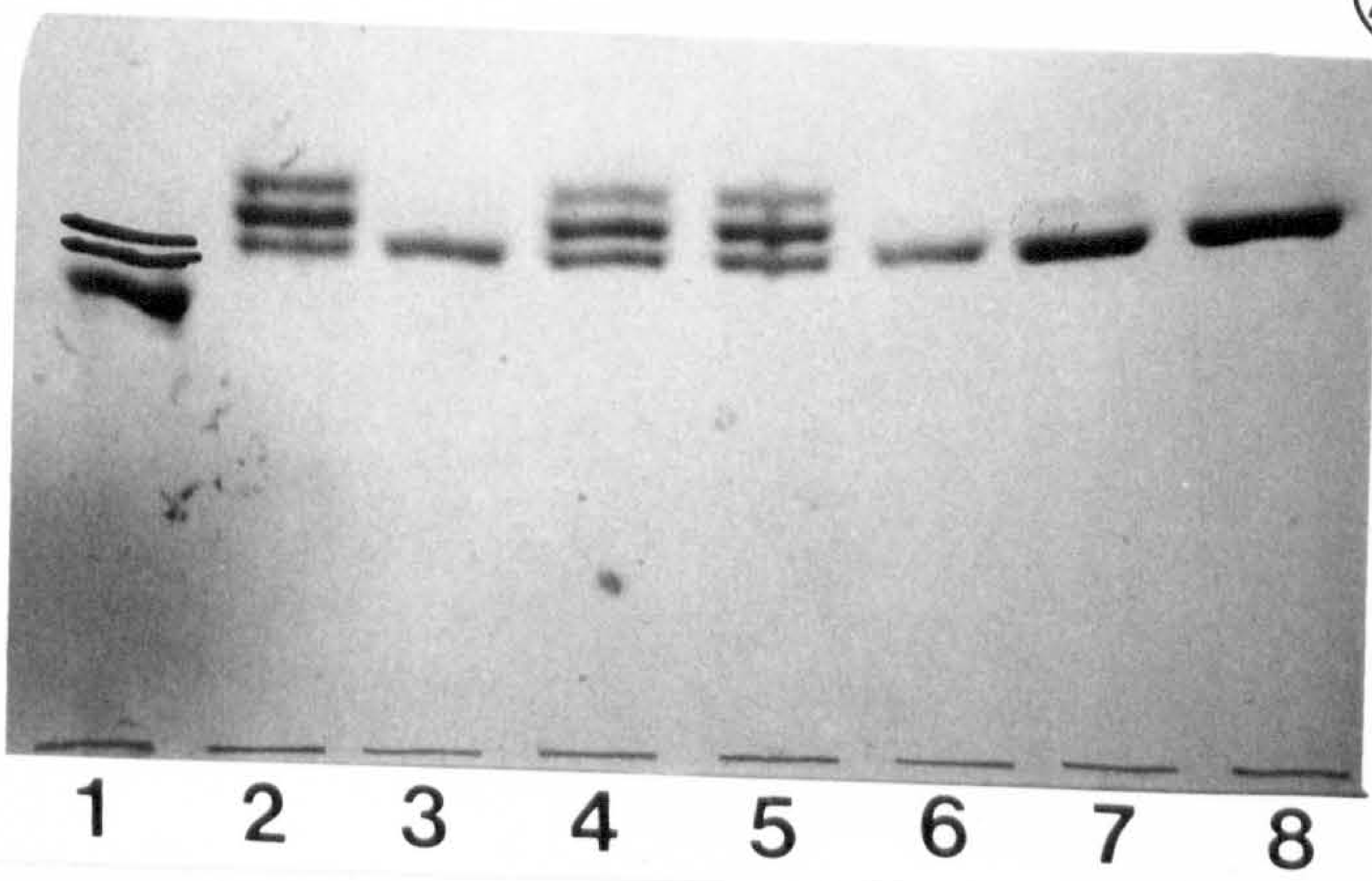
Plate 26. 6-phosphogluconate dehydrogenase (6PGD)

1. MHOM/SU/60/OD	L.tropica (LON-7)*
2. MHOM/IL/00/Singer	L.tropica (LON-10)*
3. MHOM/SA/83/Giz33	L.tropica (LON-63)*
4. ISER/SA/87/SSP286	
5 ISER/SA/87/SSP286	
6. ISER/SA/87/SSP454	
7. MHOM/SA/87/A60	
8. MHOM/SA/87/A77	

NOTE: In Fig.26 (track 1), 29 (track 8), 37 (track 7) and 38 (track 5), bands that were visible on the actual plates but were unclear on the photographs have been emphasised in ink.







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Zymograms (contd.)

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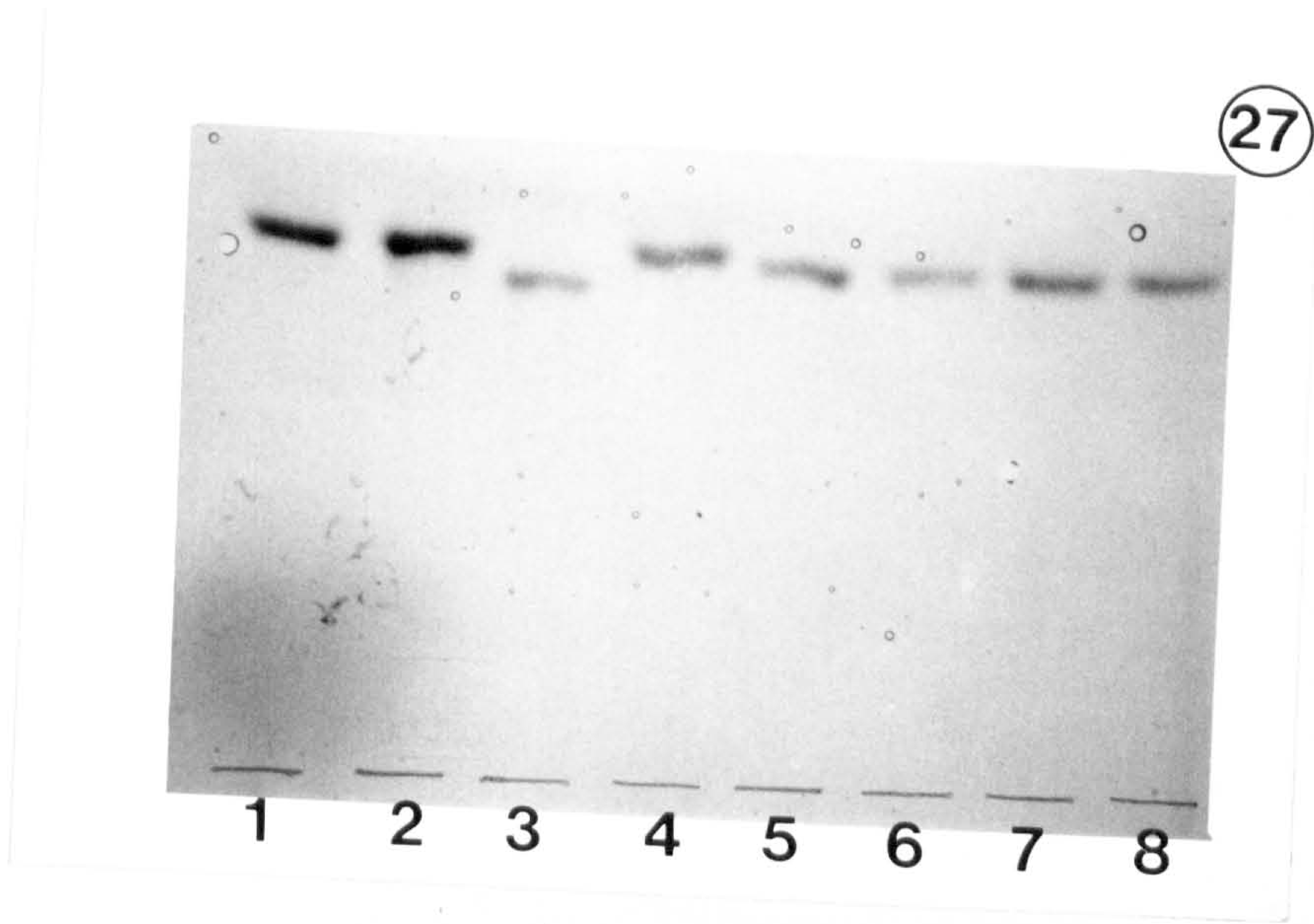
Plate 27. Phosphoglucomutase (PGM)

Samples	Identification
1. MHOM/SU/60/OD	L.tropica (LON-7)*
2. MHOM/IL/00/Singer	L.tropica (LON-10)*
3. MHOM/SA/83/Giz33	L.tropica (LON-63)*
4. ISER/SA/87/SSP286	
5. ISER/SA/87/SSP286	
6. ISER/SA/87/SSP454	
7. MHOM/SA/87/A60	
8. MHOM/SA/87/A77	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1
2
3
4
5
6
7
8

Zymograms (continued)

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Plate 28. Manosephosphate isomerase (MPI)

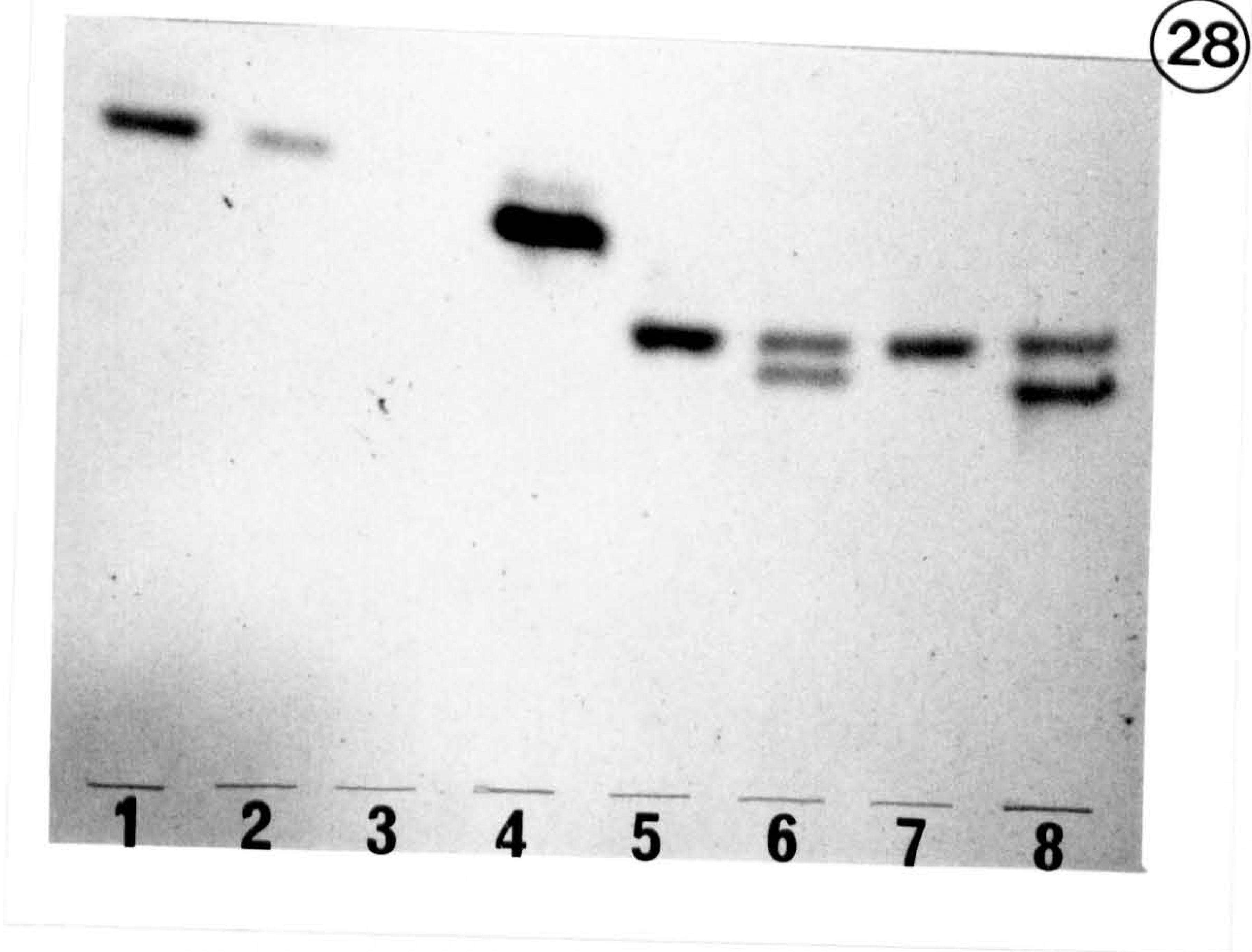
Samples	Identifications
1. MPSM/SA/83/Jish 220	L.arabica
2. MHOM/SA/84/Jish 118	L.major (LON-4)*
3. MHOM/SU/73/5ASKH	L.major (LON-1)*
4. MHOM/SA/84/Jish 118	
5. MHOM/SA/87/A25	
6. MHOM/SA/87/A29	
7. MHOM/SA/87/B1	
8. MHOM/SU/60/OD	L.tropica (Lon-7)*

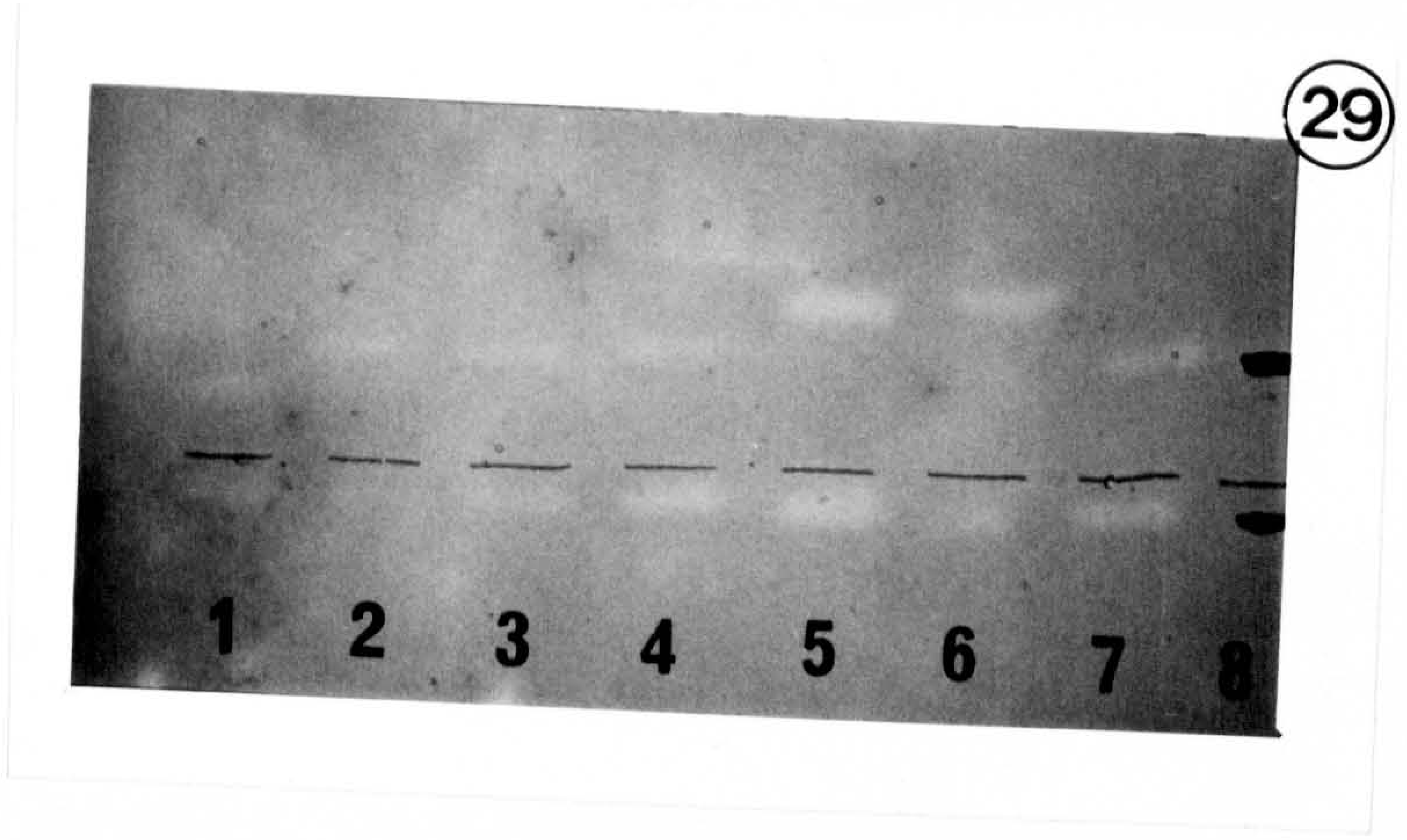
Plate 29. Superoxide dismutase (SOD)

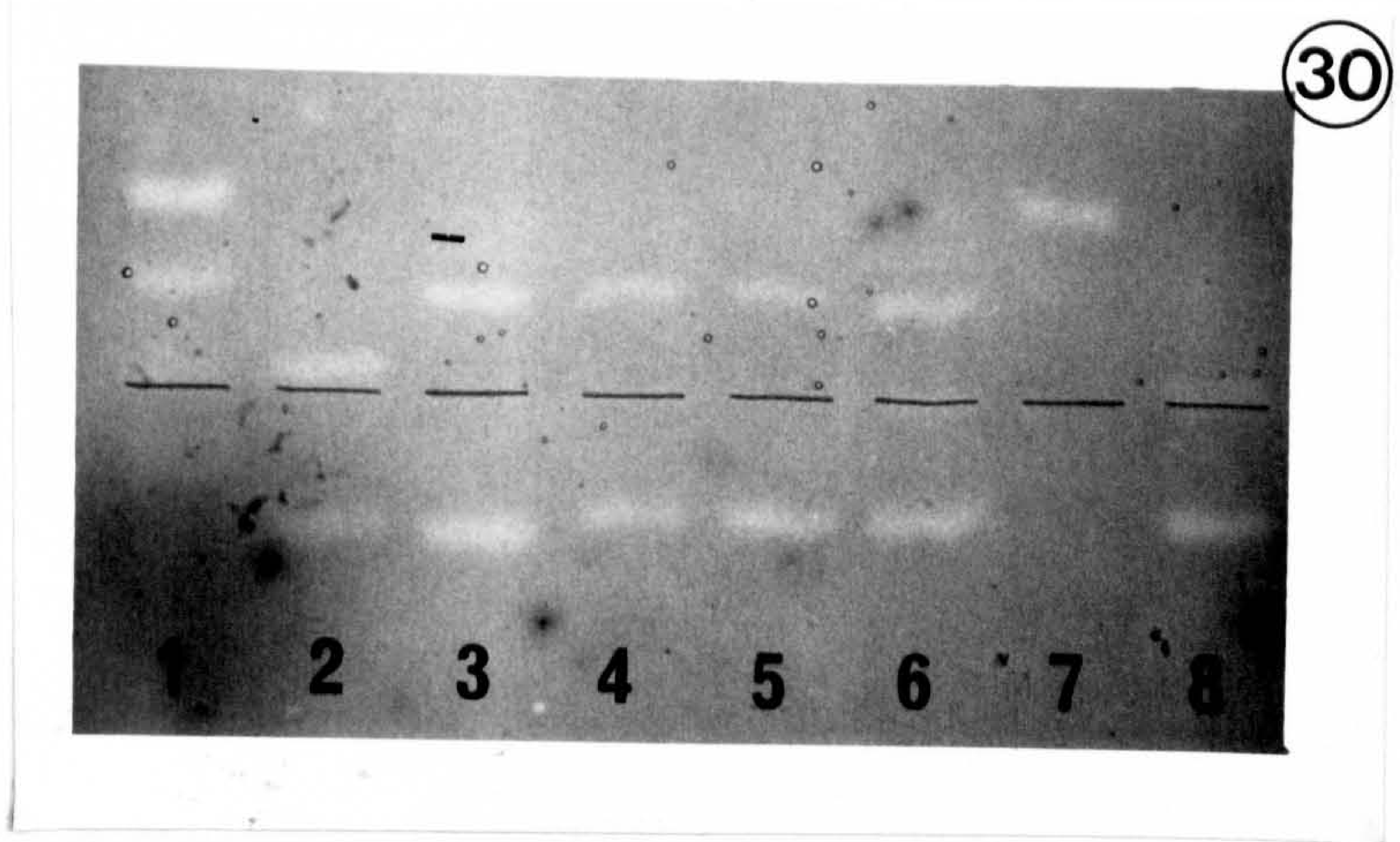
Samples	Identifications
1. MHOM/SA/83/Giz 33	L.tropica (LON-63)*
2. MHOM/SA/87/B11	
3. MHOM/SA/87/B13	
4. MHOM/SA/87/B15	
5. MHOM/IL/00/Singer	L.tropica (LON-10)*
6. MHOM/SA/87/B635	
7. MHOM/SA/87/B848	
8. MHOM/SA/87/503	

Plate 30. Superoxide dismutase (SOD)

Samples	Identifications
1. MHOM/SU/60/OD	L.tropica (LON-7)*
2. MHOM/SA/83/Giz33	L.tropica (LON-63)*
3. MHOM/SA/87/A76	
4. MHOM/SA/87/A51	
5. MHOM/SA/87/B1	
6. MHOM/SA/87/B812	
7. MHOM/SA/87/A127	
8. MHOM/SA/87/A129	







221

Zymograms (continued)

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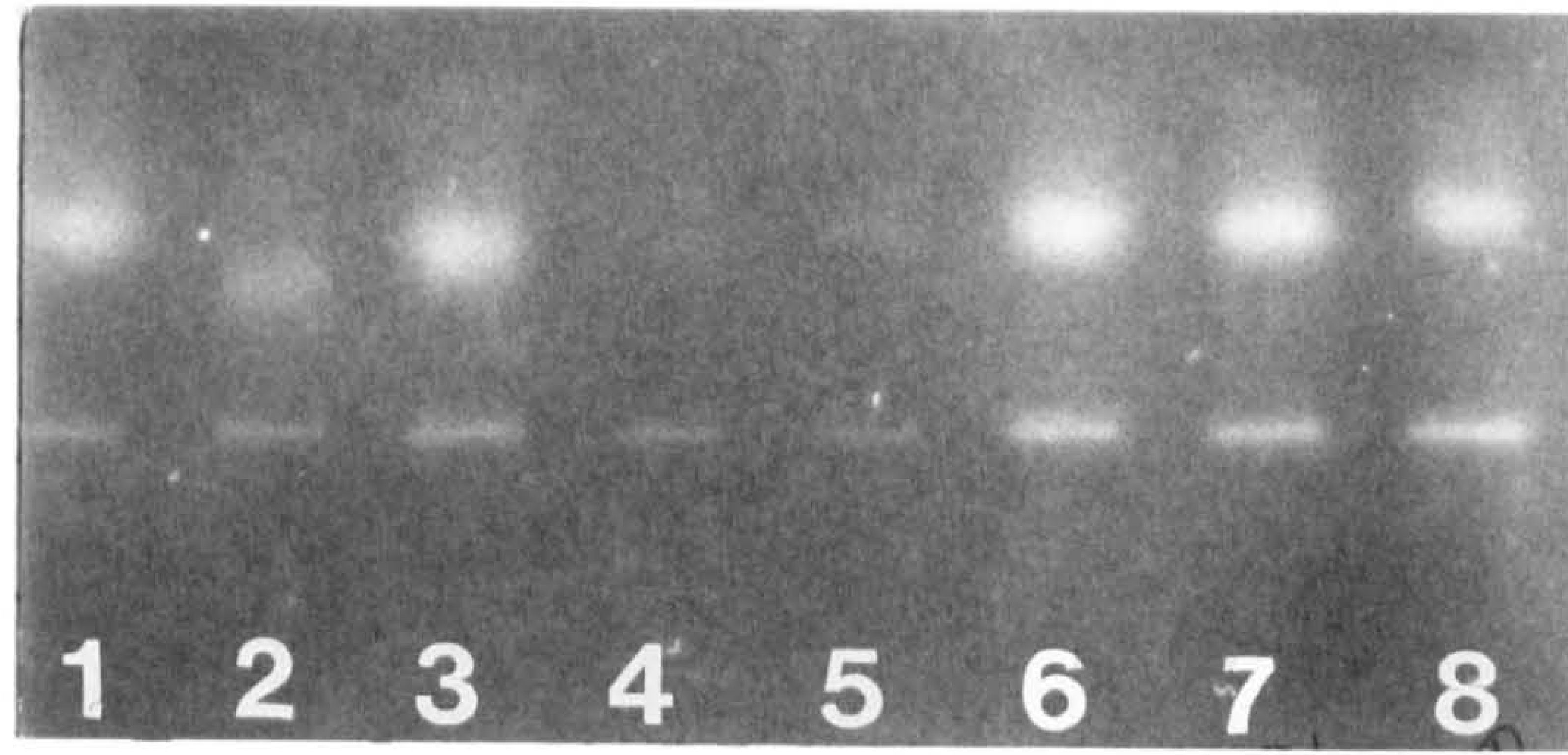
Plate 31. Estrase (ES)

Samples	Identification
1. MHOM/SU/73/5ASKH	L.major (LON-1)*
2. MHOM/SA/184/Jisha 118	L.major (LON-4)*
3. MHOM/SU/60/OD	L.tropica (LON-7)*
4. MHOM/SA/87/A65	
5. MHOM/SA/87/B16	
6. MHOM/SA/87/B17	
7. MHOM/SA/87/A122	
8. MHOM/SA/87/A69	

Plate 32. 6-phosphogluconate dehydrogenase (6PGD)

1. MHOM/SU/73/5ASKH	L.major (LON-1)*
2. MHOM/SA/84/Jisha 118	L.major (LON-4)*
3. MPSM/SA/84/Jisha 224	L.arabica
4. MPSM/SA/84/Jisha 224	L.arabica
5. MPSM/SA/83/Jisha 220	L.arabica
6. MHOM/SA/87/A53	
7. MHOM/SA/87/A59	
8. MHOM/SU/60/OD	L.tropica (LON-7)*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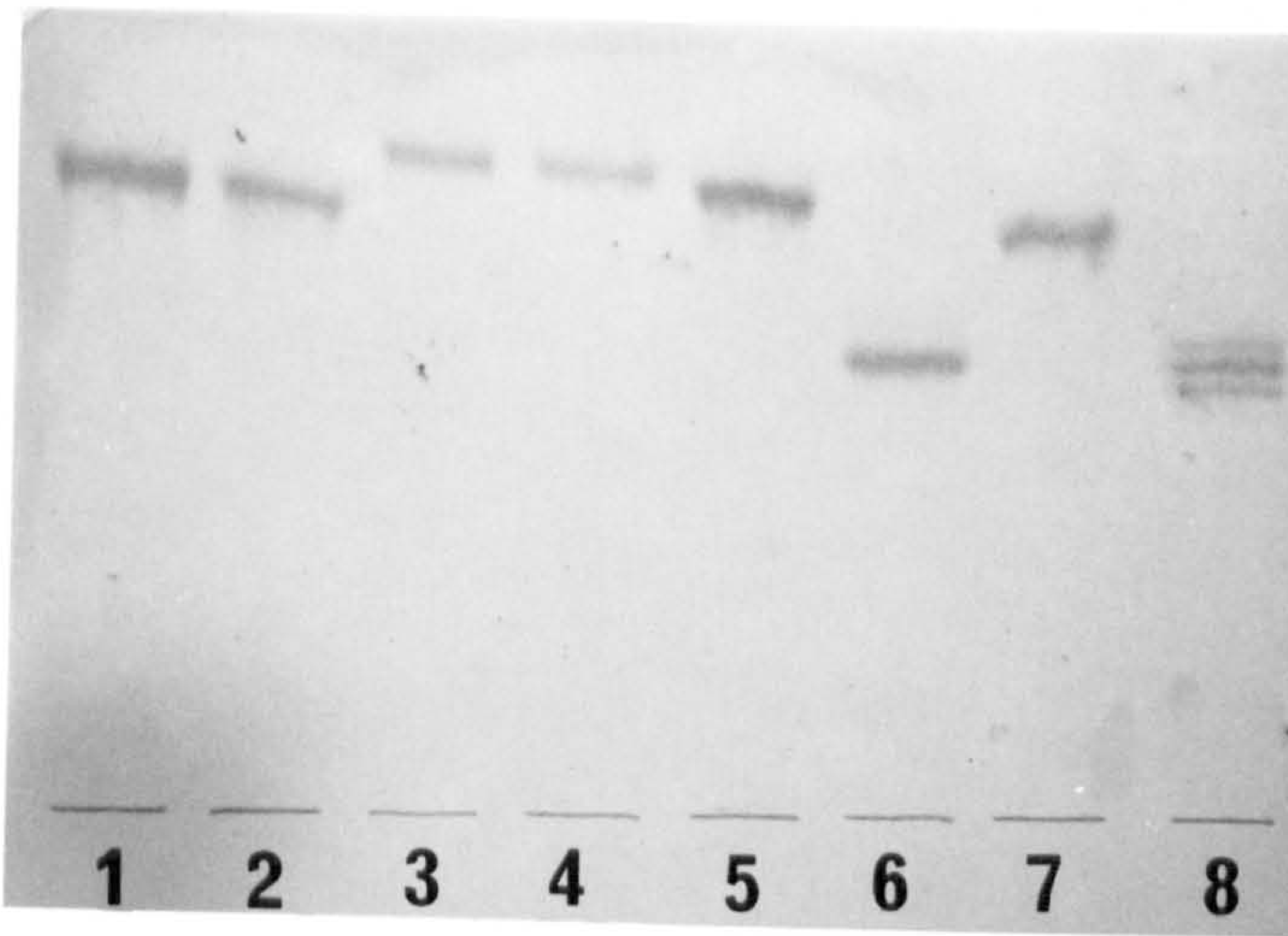
1 2 3 4 5 6 7 8


32
1 2 3 4 5 6 7 8

Zymograms (continued)

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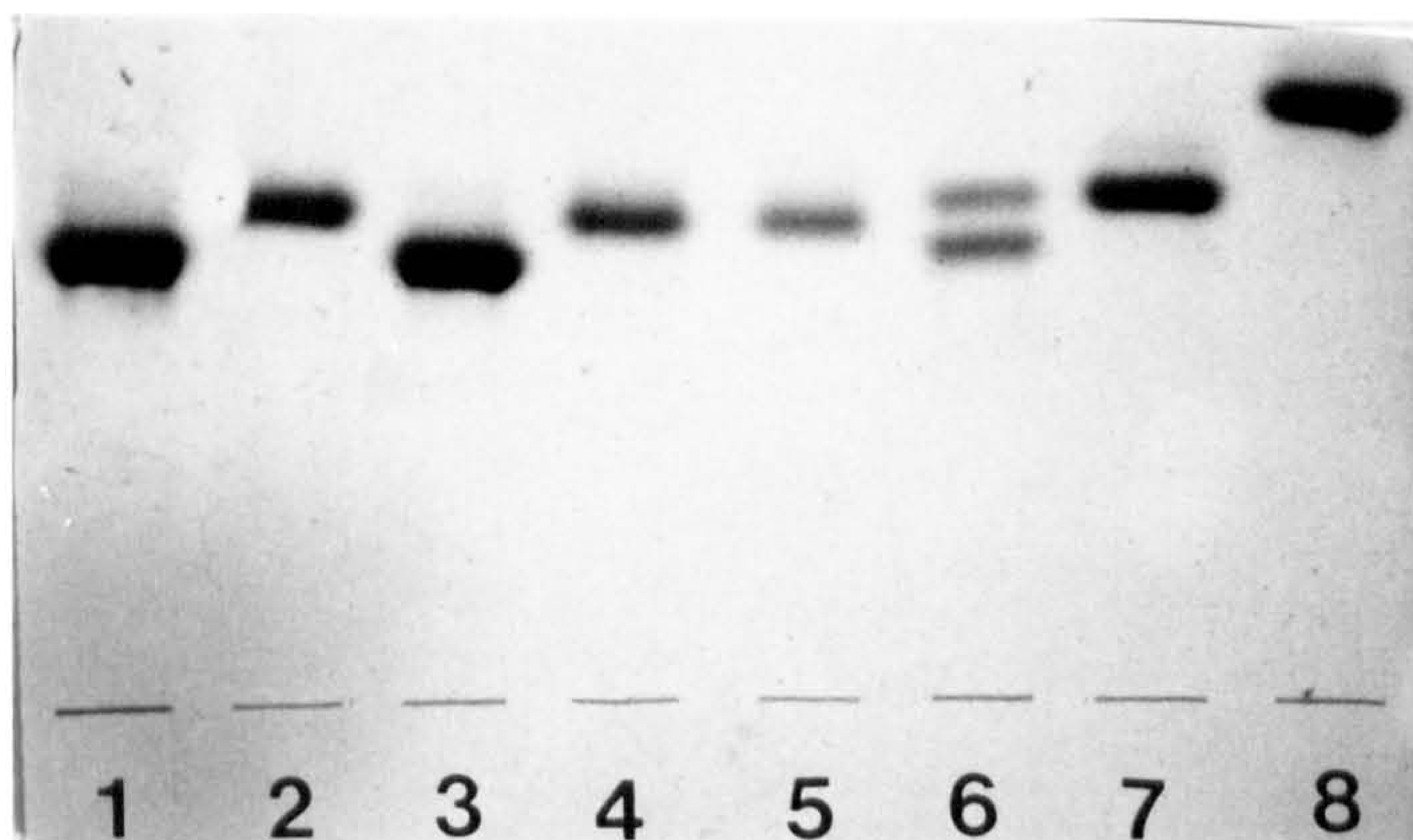
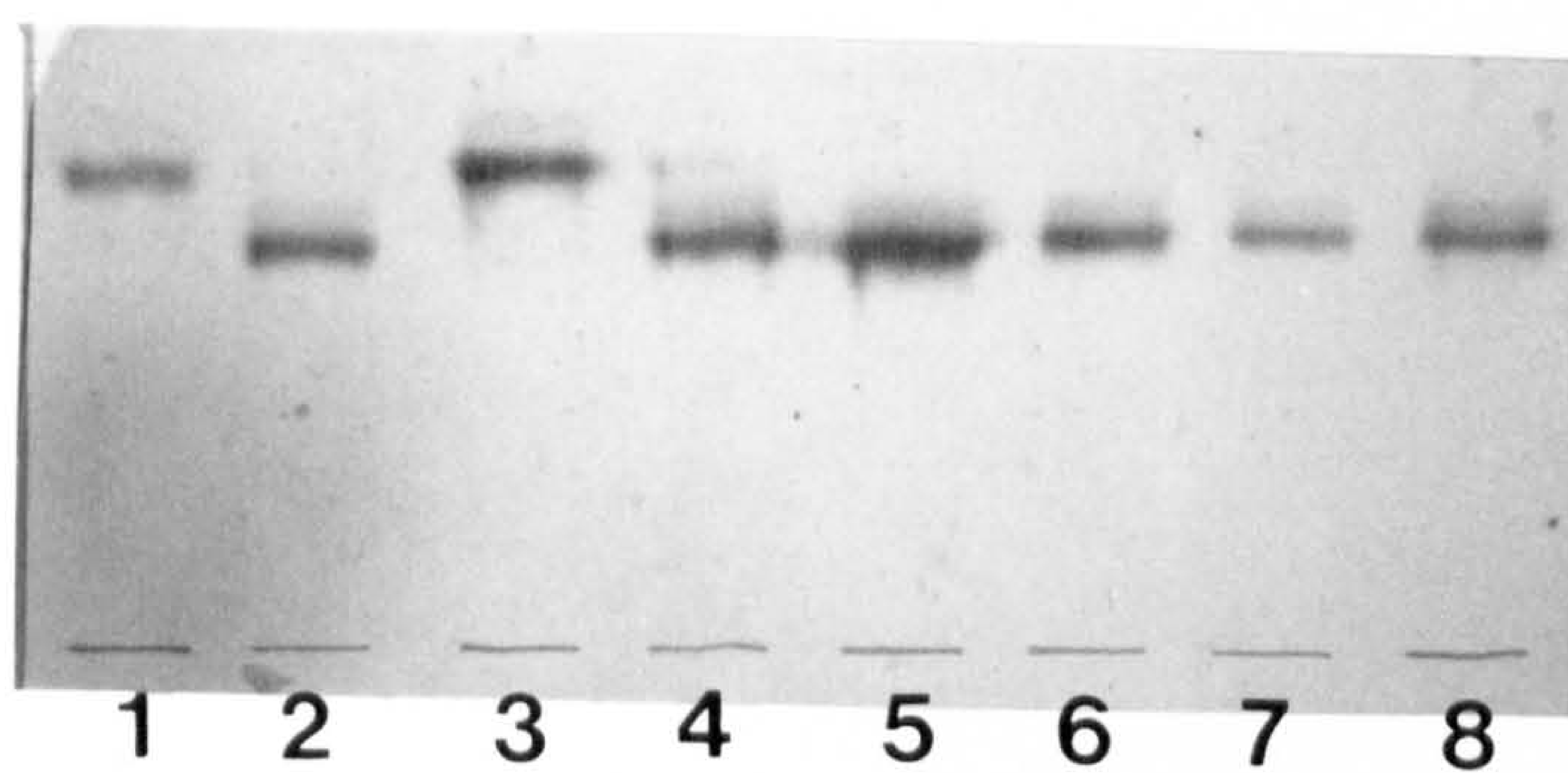
Plate 33. Manose phosphate isomerase (MPI)

Samples	Identification
1. MHOM/IN/80/DD8	*L.donovani* (LON-41)*
2. MHOM/SA/Jeddah-KA	*L.donovani* (LON-42)*
3. MHOM/TN/80/IPT1	*L.infantum* (LON-49)
4. MHOM/SA/87/VL6	
5. MHOM/SA/87/VL29	
6. MHOM/SU/60/OD	*L.tropica* (LON-7)*
7. MHOM/SA/87/B812	
8. MHOM/SA/87/A69	

Plate 34. Glucose phosphate isomerase (GPI)

1. MHOM/IN/80/DD8	*L.donovani* (LON-41)
2. MHOM/SA/81/Jeddah-KA	*L.donovani* (LON-42)
3. MHOM/TN/80/IPT1	*L.infantum* (LON-49)
4. MHOM/SA/87/VL20	
5. MHOM/SA/87/VL23	
6. MHOM/SA/87/VL27	
7. MHOM/SA/87/VL35	
8. MHOM/SA/87/VL44	





225

Zymograms (continued)

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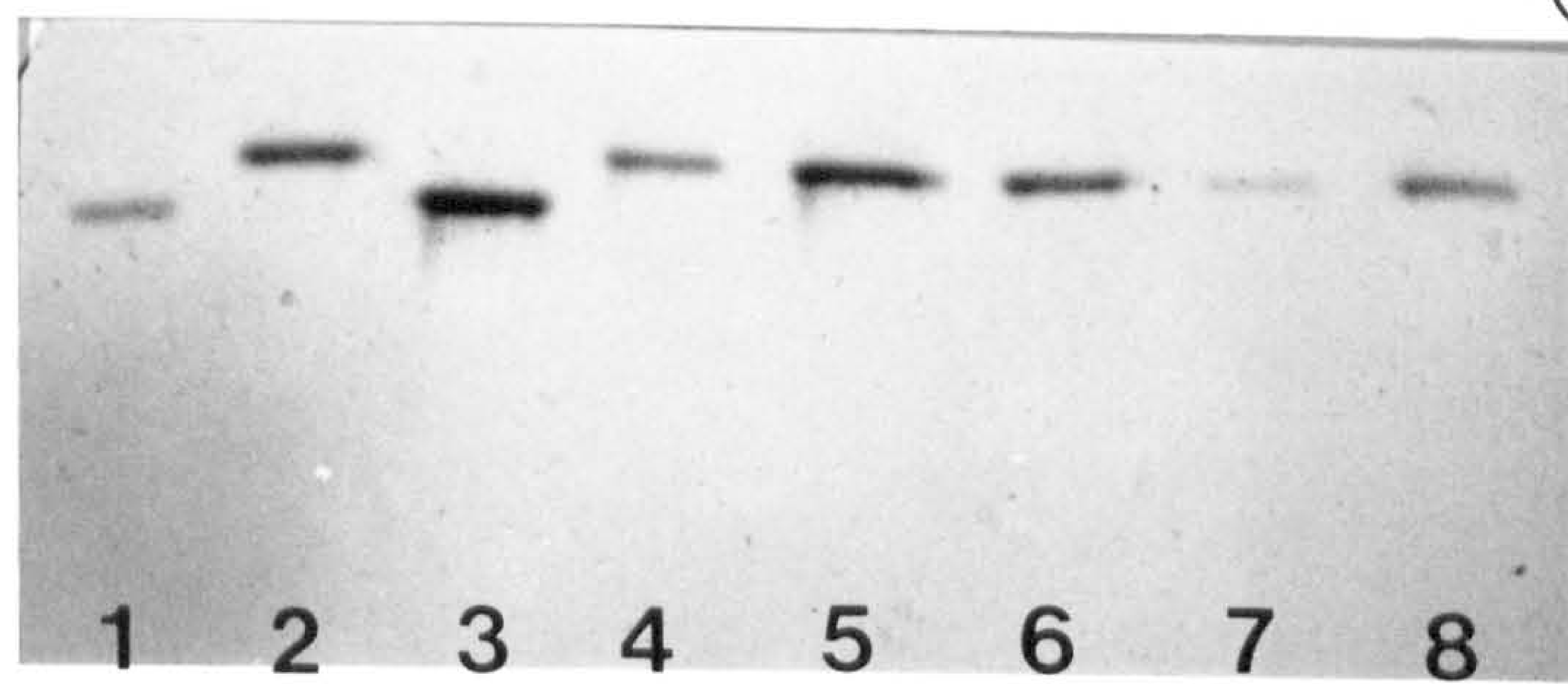
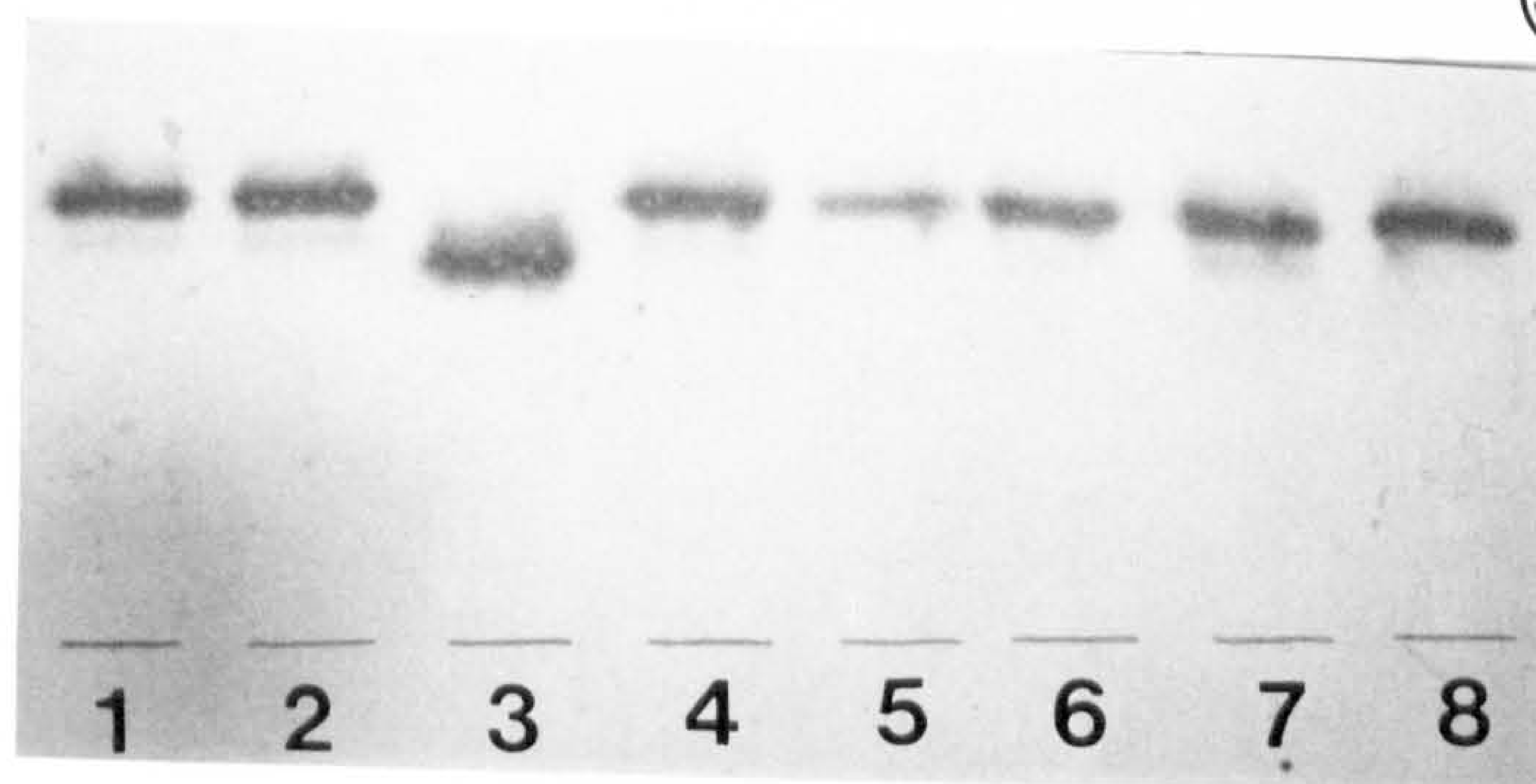
Plate 35. Mannose phosphate isomerase (MPI)

Samples	Identification
1. MHOM/IN/80/DD8	*L.donovani* (LON-41)
2. MHOM/SA/81/Jeddah-KA	*L.donovani* (LON-42)
3. MHOM/TN/80/IPT1	*L.infantum* (LON-49)
4. MHOM/SA/87/VL20	
5. MHOM/SA/87/VL23	
6. MHOM/SA/87/VL27	
7. MHOM/SA/87/VL35	
8. MHOM/SA/87/VL44	

Plate 36. Glucose phosphate isomerate (GPI)

1. MHOM/IN/80/DD80	*L.donovani* (LON-41)
2. MHOM/Et/67/HU3	*L.donovani* (LON-46)
3. MHOM/SA/81/Jeddah-KA	*L.donovani* (LON-42)
4. MCAN/SA/87/D73S	
5. MCAN/SA/87/D76S	
6. MCAN/SA/87/D81S	
7. MHOM/TN/80/IPT1	*L.infantum* (LON-49)
8. MHOM/TN/80/IPT1	*L.infantum* (LON-49)





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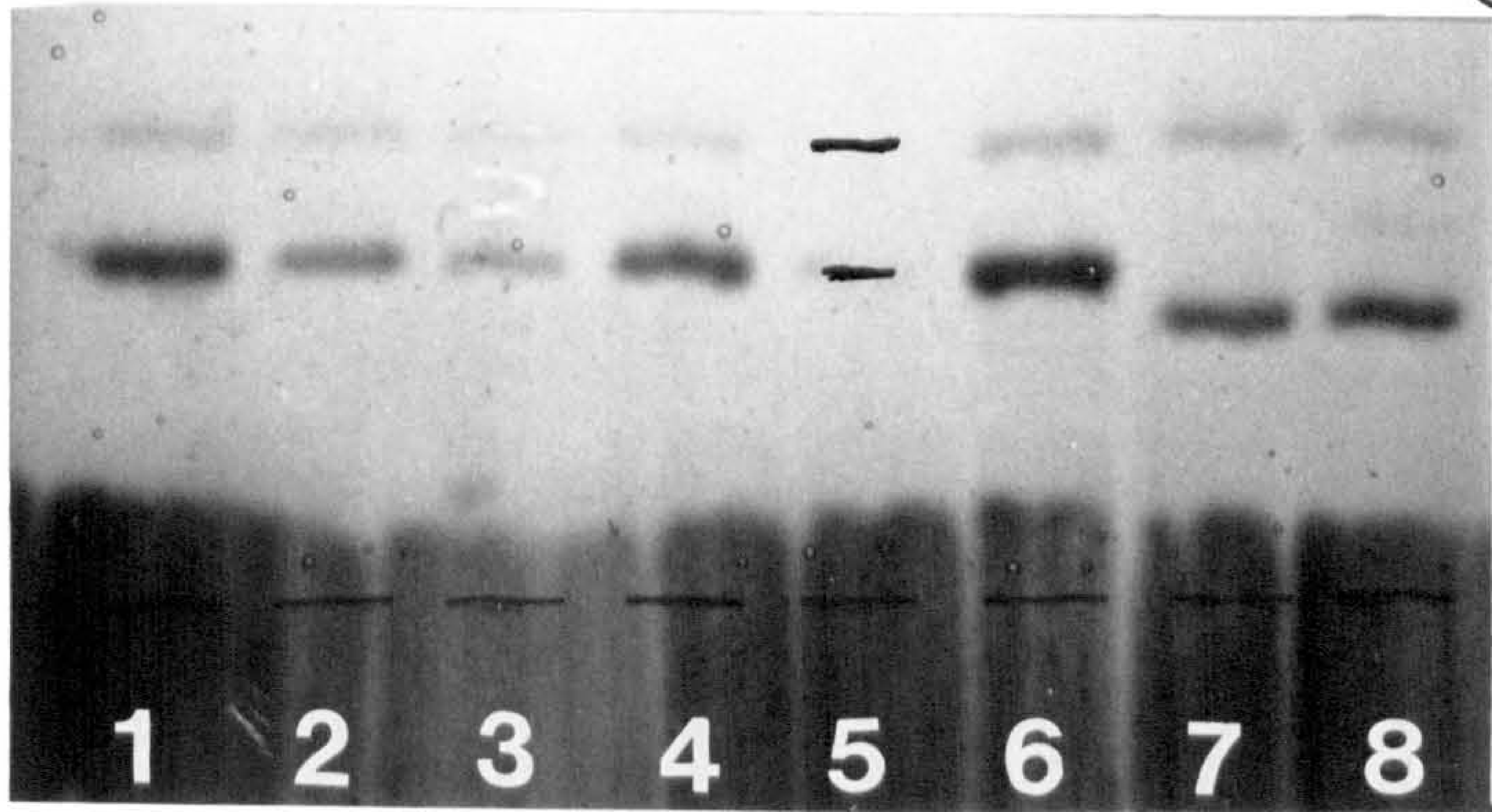
Zymograms (continued)

Plate 37. 6-phosphogluconate dehydrogenase (6PGD)

Samples	Identification
1. MHOM/SU/60/OD	L.tropica (LON-7)
2. MHOM/SA/00/Stiehl	L.tropica (LON-22)
3. MHOM/IQ/73/A.Sinai III	L.tropica (LON-11)
4. MHOM/SA/87/B1	
5. MHOM/SA/87/A25	
6. MHOM/SA/87/A32	
7. MHOM/SA/87/A36	
8. MHOM/SA/87/B3	

Plate 38. Malate dehydrogenase (MDH)

1. MHOM/IL/00/Singer	L.tropica (LON-10)
2. ISER/SA/87/SSP286	
3. ISER/SA/87/SSP454	
4. MHOM/SA/87/A126	
5. MHOM/SA/87/A127	
6. MHOM/SU/73/32ASKH	L.tropica (LON-18)
7. MHOM/SA/83/Giz33	L.tropica (LON-63)
8. MHOM/SA/87/A130	



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Part 3: Studies on visceral leishmaniasis

1. Kala-azar

1.1 Cultures

A total of 60 tubes of NNN culture medium were inoculated with bone marrow specimens from kala-azar patients at Mahayel Hospital in Asir province and King Fahad Hospital (KFH) in Gizan province.

The cultures were transferred to the Abha laboratory every week from Mahayel and every two weeks from Gizan for follow-up.

20 out of 60 cultures were positive (33%). Age, nationality and localities of the patients with positive cultures are given in Table 25. The cultures were positive within an average of 14 days and the negative cultures were discarded only after 6 weeks follow-up.

The majority of positive cultures were from the Gizan province where better facilities were available to obtain marrow aspirates from the patients (see Table 25).

1.2 Isoenzyme typing

Isolates of visceralising *Leishmania* were characterized by the isoenzyme method using up to 12 enzymes. The samples were run side by side with three reference strains of profiles LON-41 (MHOM/IN/80/DD8), LON-49 (MHOM/TN/80/IPT1) and LON-42 (MHOM/SA/81/Jeddah-KA).

All the samples corresponded to the reference strain of *L.donovani sensu lato*, profile LON-42, which differs from

TABLE 25 Summary data on isolates from patients with visceral leishmaniasis.

Isolate Designation:	Age (years)	Nationality	Locality Emirate	Locality Sub-Emirate (District)	Bone marrow smear
MHOM/SA/87/VL6	1.5	Saudi	Mahayel	Kamis mutar	+
MHOM/SA/87/VL16	0.8	Saudi	Mahayel	Bani-Thwa	+
MHOM/SA/87/VL20	-	Saudi	Gizan	Bani-Malik	-
MHOM/SA/87/VL25	2	Saudi	Mahayel	Hasso	+
MHOM/SA/87/VL27	-	Saudi	Mahayel	-	+
MHOM/SA/87/VL29	-	Saudi	Gizan	Abu-aresh	+
MHOM/SA/87/VL35	-	Saudi	Gizan	Al_Ahid	?
MHOM/SA/87/VL44	-	Saudi	Gizan	Abu-aresh	+
MHOM/SA/87/VL48	-	Saudi	Mahayel	Mahayel	+
MHOM/SA/87/VL51	24	Yemeni	Gizan	Al-Khoba*	+
MHOM/SA/87/VL52	3	Saudi	Gizan	Aeban	+
MHOM/SA/87/VL54	6	Yemeni	Gizan	Al-Ahid*	+
MHOM/SA/87/VL55	13	Yemeni	Gizan	Al-Ahid	-
MHOM/SA/87/VL56	1.5	Saudi	Gizan	Aeban	-
MHOM/SA/87/VL57	2	Saudi	Gizan	Al-Khoba	-
MHOM/SA/87/VL58	4	Yemeni	Gizan	Abu-aresh	+
MHOM/SA/87/VL59	4	Yemeni	Gizan	-*	+

Notes:

*The patient came only for treatment, from Yemen

The identity of the parasites of all the isolates was Leishmania donovani sensu lato Zymodeme (LON-42)

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LON-41 in five enzymes (MDH, 6PGD, MPI, GPI, PEPD) and from LON-49 in six enzymes (MDH, ASAT, MPI, GPI, PEPD, NH) as reported by Le Blancq and Peters (1986a), (see plates 33 - 35). Surprisingly, the human VL isolates were different from the dog isolates collected from the same area which were typical L.infantum zymodeme (LON-49), (see map 11). The human VL isolates originated from different localities, including the Al-Khoba area where the isolates from dogs were collected.

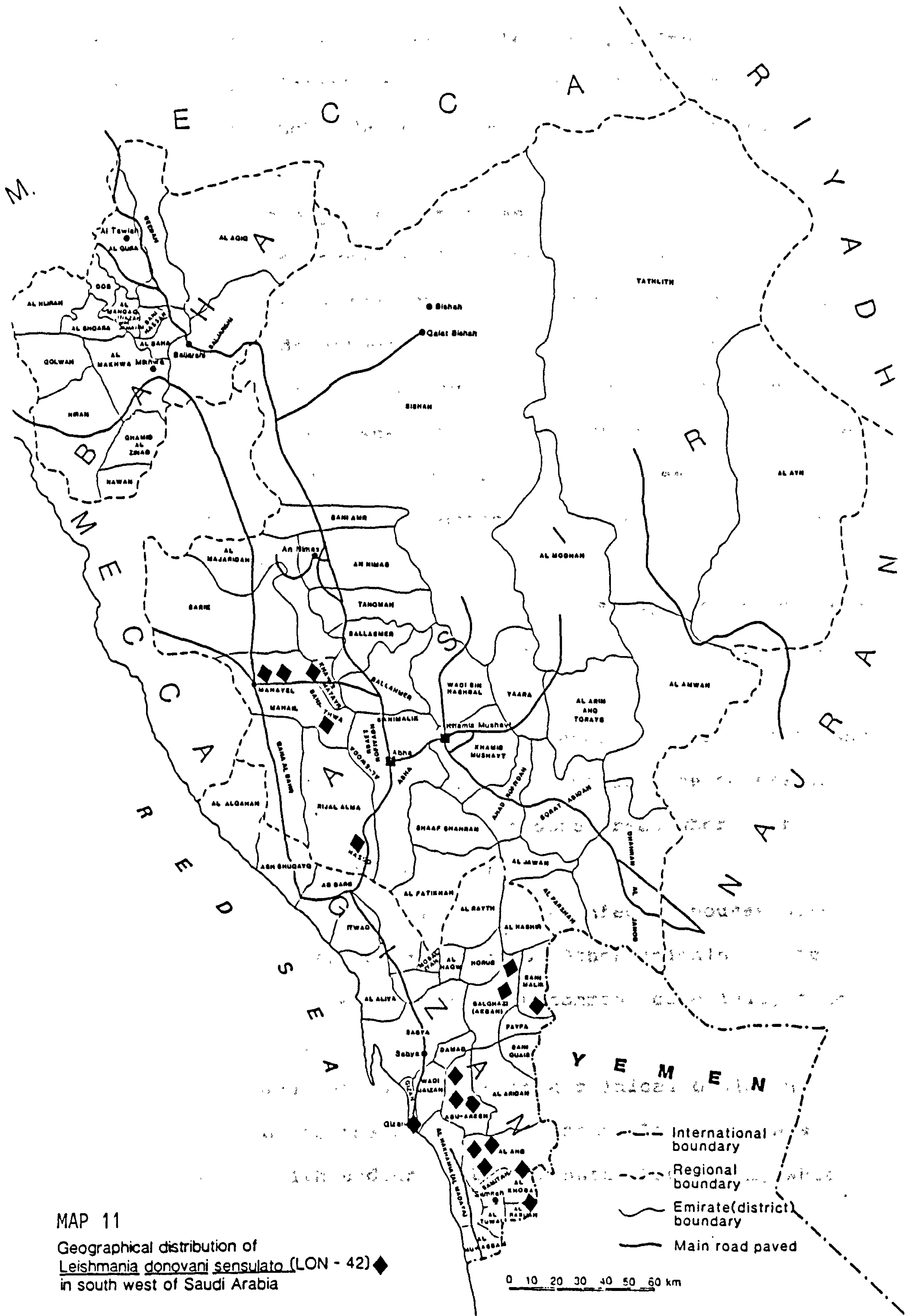
Three isolates (MHOM/SA/87/VL51, MHOM/SA/87/VL54 and MHOM/SA/87/VL59 were from Yemeni patients who came to Gizan province to seek treatment but were not residents.

1.3 Blood survey of children

Twenty six (3.7%) out of 706 children's blood spots were ELISA positive [positive value was determined to be 0.41 optical density or higher at 492 nm upwards (Voller, 1988, personal communication)]. The numbers examined with the ELISA values from each location are shown in Figure 20.

Among the ELISA positive, 42% were at the lower limit of positivity, value 0.4 - 0.5 OD and only one child gave a highly positive (>1.0) OD value, see Figure 21.

The number of children examined in each age group and the percentage of positive ELISA values for a given age are presented in Figure 22, which shows that the highest percentages are between the ages 3-4 and 0-1 years. However, the relationship between age and ELISA seropositive



MAP 11

Geographical distribution of _Leishmania donovani sensulato_ (LON - 42) ◆ in south west of Saudi Arabia

values (Figure 23) was tested statistically using a correlation coefficient ($r = 0.0026$; $p = 0.99$) and this showed no relationship between the age and ELISA value.

1.4 <u>Investigation of kala-azar cases</u>

Using the case investigation form (see annex 1) more than 50 cases of kala-azar were investigated by the Abha and Gizan <u>Leishmania</u> departments. 50 case investigation forms were analysed and the following conclusions can be drawn up:

(i) Most of the cases were originally from houses located on hills near the valleys (dry with seasonal running water) some of the houses are situated on the sides of these valleys.

(ii) Infected houses which were visited by the writer were in a poor condition and the standard of hygiene was very low.

(iii) The climate of the kala-azar endemic area is hot, and the local people often sleep outside the houses or in open door rooms, except in some areas where air conditioning is possible.

(iv) The abundant animals near the infected houses were goats, sheep, cats and dogs. Other animals, either domestic or wild, are not uncommon, especially foxes and wolves.

(v) All cases were diagnosed on a clinical basis in addition to the bone marrow smear. Treatment was mainly with sodium stibogluconate (Pentostam) which

is available in all the hospitals in endemic areas. An important factor in evaluation of treatment and the follow-up of patients is that the parents of some children refused to continue their treatment in the hospitals or even through the nearest primary health care centre after the first few injections.

(vi) The direct follow-up of all patients could not be fully investigated as regards history of the disease and laboratory and clinical investigations. However, generally speaking, multiple infections in single houses were not reported. No attempt was made to carry out a mass seroepidemiological survey to determine the cryptic infection rate other than the sampling made during this study which was reported above (part 1.3 of this chapter).

(vii) The sandfly species collected from the homes of kala-azar patients are reported in the section on entomological studies, (see Tables 34 & 35). Apart from the genus Sergentomyia, among the genus Phlebotomus, Ph.bergeroti was the dominant species, followed by Ph.sergenti, then Ph.alexandri and Ph.papatasi. In spite of the high number of female Phlebotomus dissected from kala-azar endemic areas of the lowlands (see Table 37), no Phlebotomus females were infected with Leishmania promastigotes. The possible vector(s) of kala-azar in this study area are therefore still unknown.

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Fig. 20. ELISA values related to place of origin in the child epidemiological survey

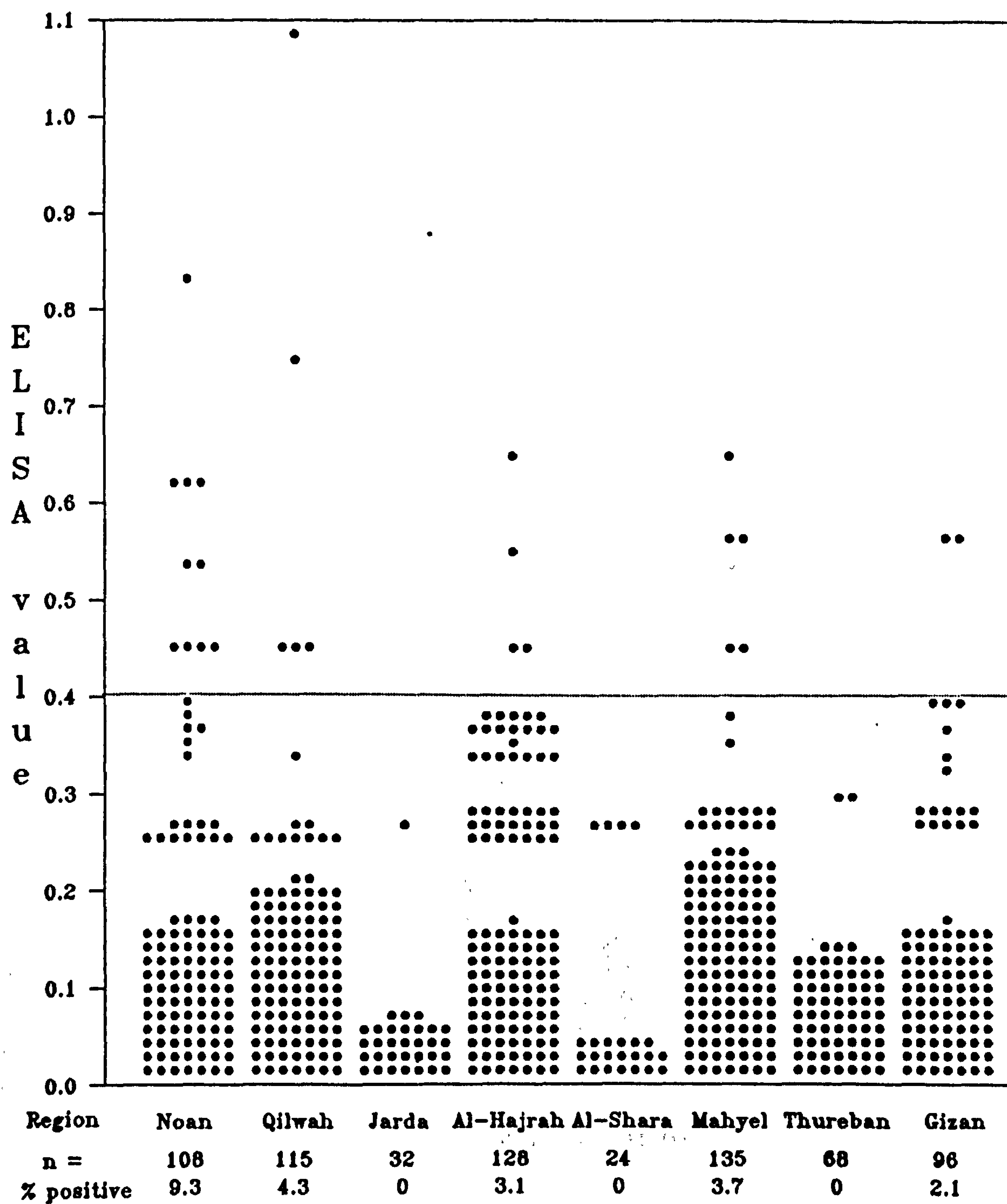


Fig. 21. Distribution of ELISA values among the ELISA positives

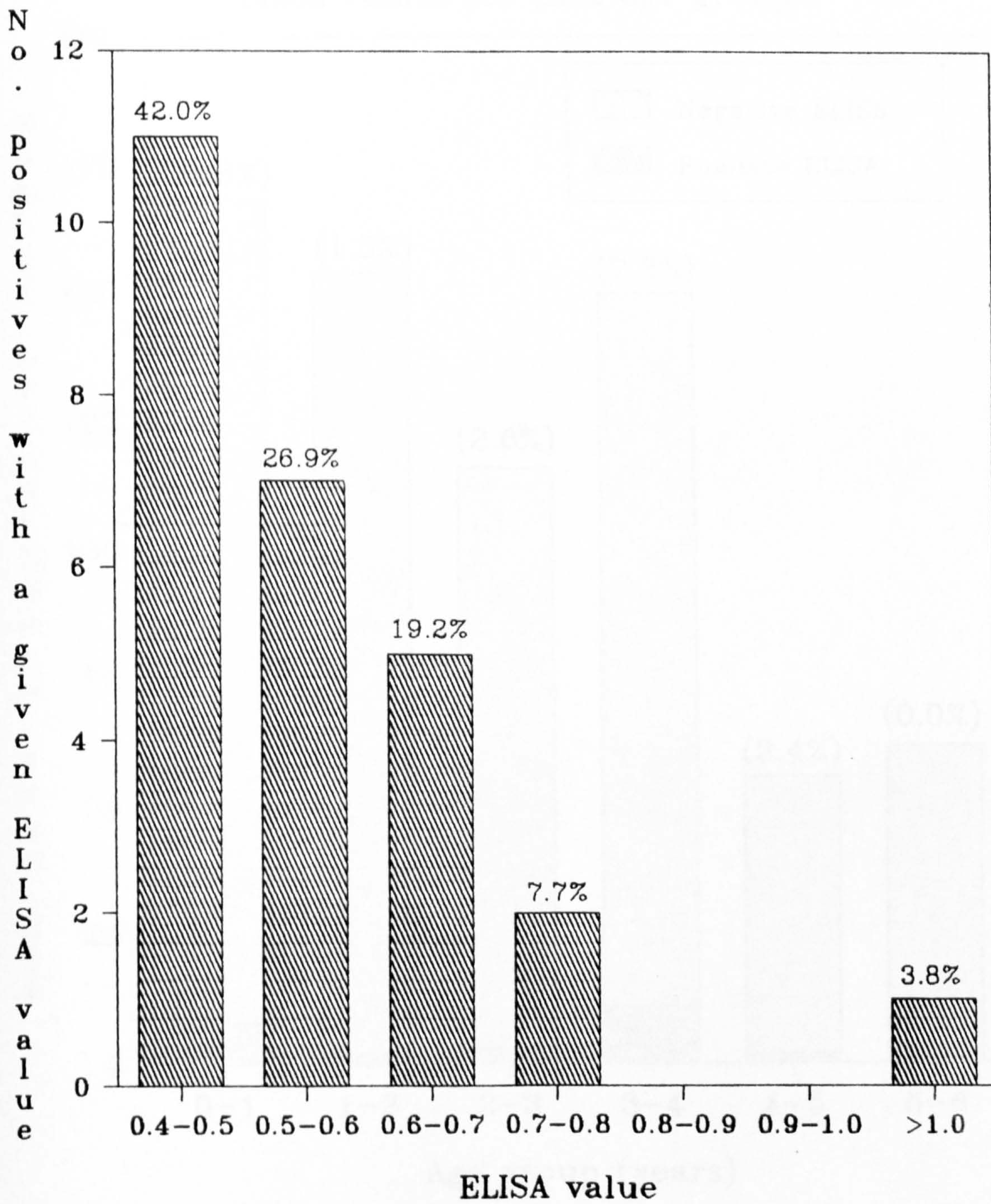


Fig. 22. The distribution of ELISA seropositive values by age

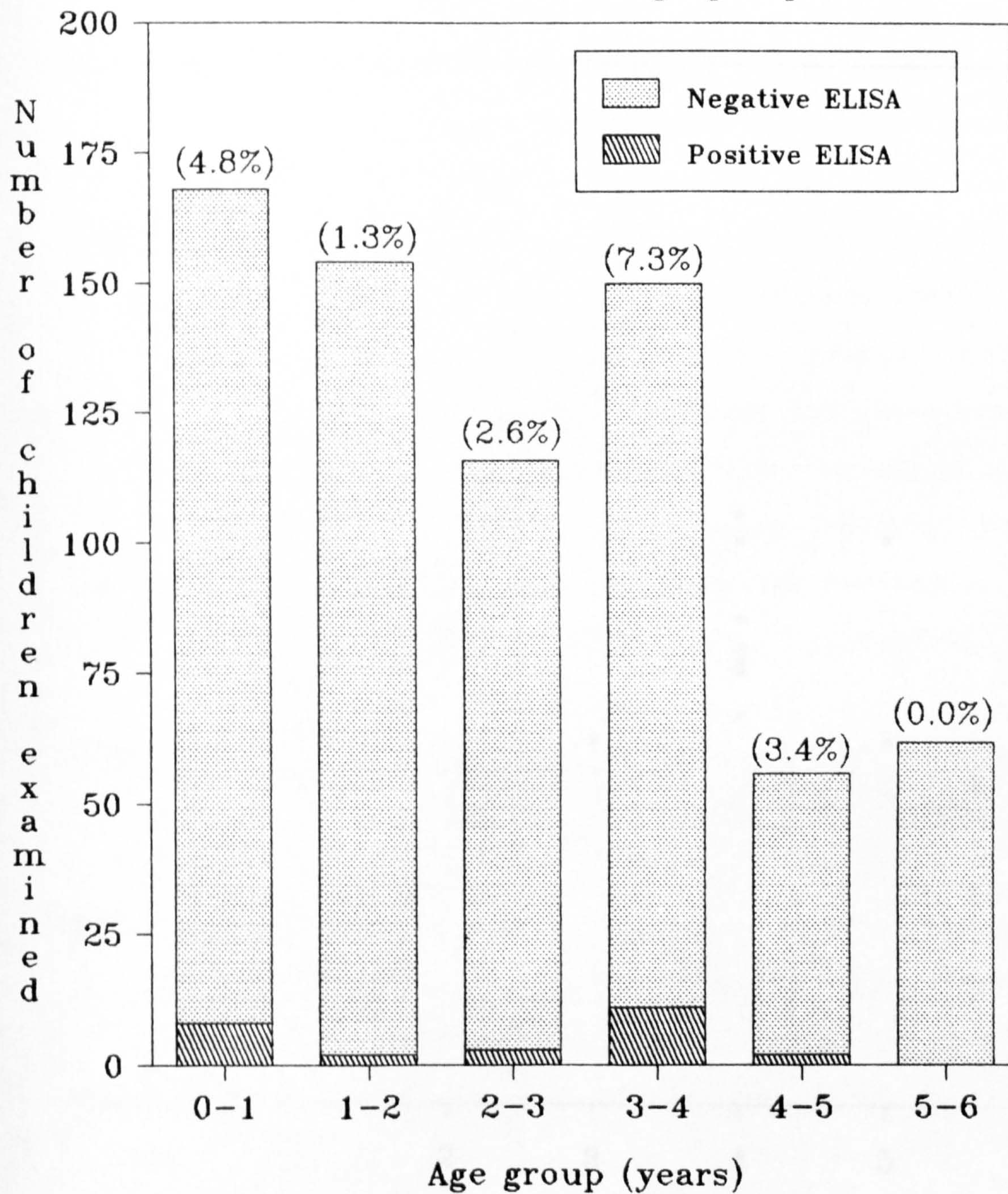
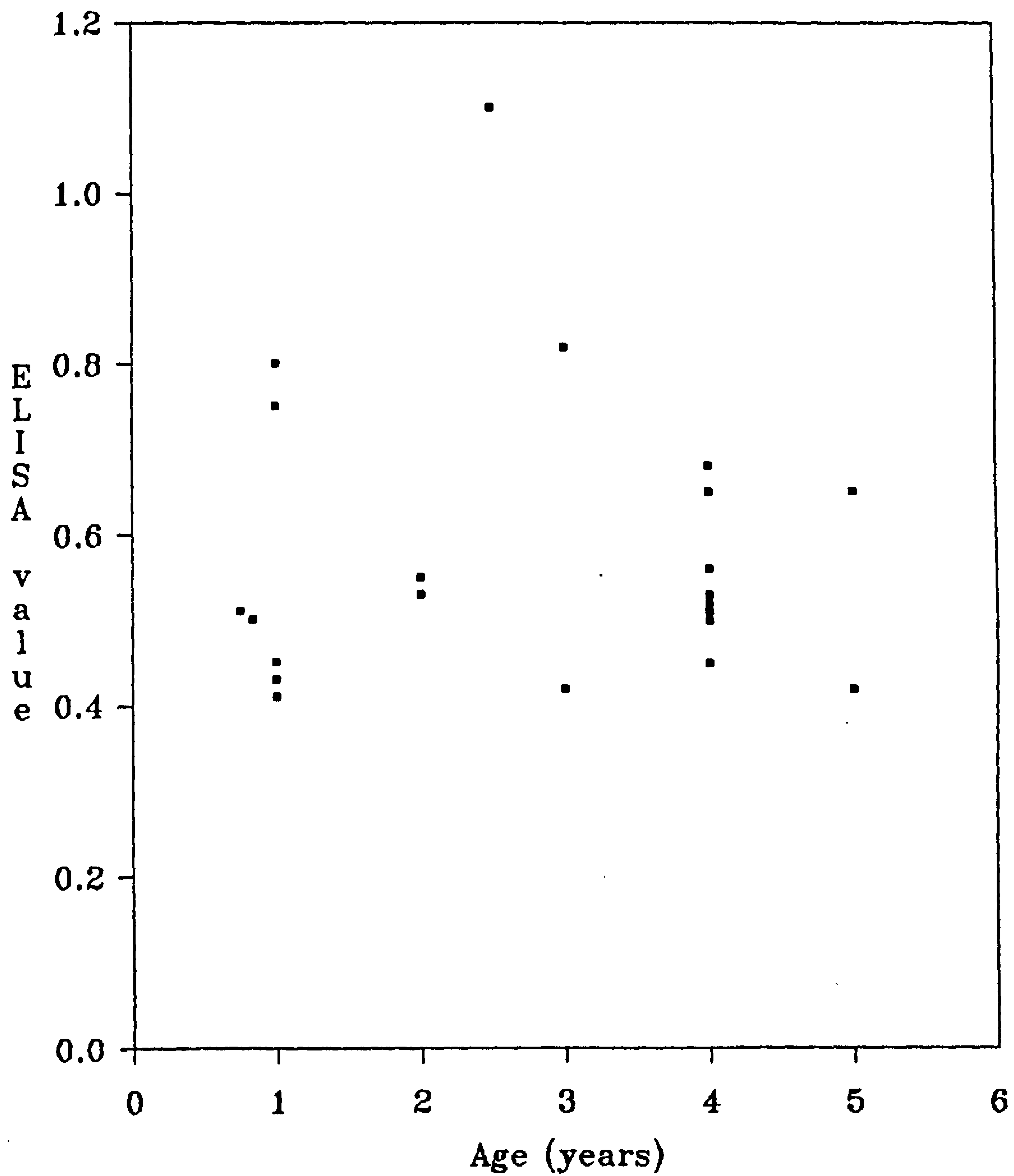


Fig. 23. The relationship between age and ELISA seropositive values



1.5 Animal inoculation

For primary isolation only two hamsters were injected by bone marrow specimens directly from the patient. Both hamsters died after six weeks and the spleen and liver cultures were negative. Due to difficulties with the animal facilities in the hospitals, direct inoculation for primary isolation was stopped.

For biological filtration from contaminated cultures (VL27, VL29, VL44 and VL48), material was inoculated into hamsters. Three to four months later the hamsters were killed by anaesthesia and dissected. Spleen and liver cultures were positive in three hamsters injected with V129, VL44 and VL48. The cultures were injected into hamsters as an extra safeguard in case there should be for any failure to keep the original contaminated cultures. However, the previously mentioned cultures grew well and remained clean after several subcultures with 50 ul/ml of gentamycin.

2. Studies on Dogs (Canis familiaris)

2.1 Physical examination

The results of the physical examination of each individual dog from both study areas are in Annex 3 and 4. The summary of this result is in Table 26. 22.5% and 6.7% of these dogs had splenomegaly and hepatomegaly respectively. Only three dogs, D19, D76 and D81 were emaciated and physically sick (plate 39).

2.2 Parasitological examination

2.2.1 Impression smears

Impression smears from liver and spleen of 89 dogs were examined and 6 animals were found to have parasites in their livers and/or spleens. Two of the positives were very difficult to spot (plate 40).

2.2.2 Tissue sections

Tissue sections from the livers and spleens of 89 dogs were stained by the immunoperoxidase technique and only those dogs that were positive in impression smears were also positive in tissue sections, (see Table 26). The advantages of using this technique are to confirm the result and to allow easy and unequivocal parasite identification by the distinctive yellow coloration of the amastigotes in infected macrophage cells (plates 41, 42).

2.2.3 Cultures

Cultures from livers and spleens of the 89 dogs were made in NNN medium, two culture medium bottles being inoculated by material from the organs. Additional bottle was added for spleen aspiration from dogs Nos. D43 to 91.

The cultures were made in the field laboratory in the study area and protected from over-heating by air conditioning until they were transferred to the central laboratory in Abha city. The cultures were examined

TABLE 26 Summary of dogs examined in kala-azar endemic areas of southwest Saudi Arabia

		Physical Examination					Laboratory Examinations					
Region	Number examined	Mean Size (cm)	No. with lesions in Ear	No. with lesions in Skin	No. with Hpm	No. with Spm	No. with positive smears[1] Liver	No. with positive smears[1] Spleen	No. with positive sections[2] Liver	No. with positive sections[2] Spleen	No. with Positve Cultures[3]	No. with Positiye ELISA[4]
Mahayel Asir Province	40	42x71	5 12.5%	0 0%	0 0%	8 20%	3 7.5%	3 7.5%	3 7.5%	3 7.5%	3 7.5%	5 12.5%
Al-Khoba Gizan Province	49	50x116	1 2%	2 4%	6 12.2%	12 24.5%	3 6.1%	3 6.1%	3 6.1%	3 6.1%	3 6.1%	12 24.5%
Total	89		6 6.7%	2 2.2%	6 6.7%	20 22.5%	6 6.7%	6 6.7%	6 6.7%	6 6.7%	6 6.7%	17 19.1%

Notes:

1. The positive livers and spleens are from the same positive dogs
2. These positives are the same as in 1.
3. Cultures from Mahayel were lost due to heavy contamination. The identity of the isolates from Al-Khoba were *L. infantum* LON-49
4. Three out of five from Mahayel were parasitologically positive and three out of twelve were parasitologically positive in Al-Khoba

regularly with 3 days interval. Unfortunately, under the difficult field conditions most of the cultures from dogs were highly contaminated and some definitely positive cultures were lost due to contamination, especially those from the Mahayel area. However, the field laboratory conditions in Gizan were better; three positive cultures obtained from this area were grown in bulk successfully, and typed isoenzymatically. All three positive cultures were from the spleen.

2.2.4 Animal inoculation

The results after four months follow-up of hamster inoculated with specimens and cultures from dogs, were as follows:

(a) two out of three hamsters injected with three positive cultures from dogs Nos. D73, D76 and D81 were positive for Leishmania amastigotes in the spleen and liver.

(b) none of the hamsters injected by either macerated tissue or contaminated culture were positive.

The results of parasitological examination using different techniques are shown in Table 27.

2.3 Parasite identification

Three isolates from dogs MCAN/SA/87/D73, MCAN/SA/87/D76 and MCAN/SA/87/D81 were typed by isoenzyme typing using up to 12 enzymes. Surprisingly the parasite was a typical Leishmania infantum LON-49. This is in contrast to the human

TABLE 27 Results of parasitological examination of dog samples using different diagnostic techniques.

Diagnostic Technique:	Study Areas			
	Bani-Thwa, Mahayel		Al Khoba, Gizan	
	No. examined	No. positive	No. examined	No. positive
A. Giemsa staining of impression smear				
-Liver	40	2	49	3
-Spleen	40	3	49	3
B. Immunoperoxidase staining of tissue				
-Liver	40	3	49	3
-Spleen	40	3	49	3
C. Culture				
-Liver	40	0	49	0
-Spleen	40	3*	49	2
-Spleen aspiration	-	-	49	1
D. Animal inoculation				
-From culture	4	0	3	1
-Directly by macerated tissues of either liver or spleen	3	0	2	0
Total number of positive dogs by one or more of the diagnostic methods	3 (7.5%)		3 (6.1%)	
Total number of positive dogs in both study areas	6 (6.1%)			

*A very few promastigotes were seen, but the culture was highly contaminated and could not be maintained.

Feral dog

Plate 39. A feral dog seen near habitations in a Kala-azar endemic area

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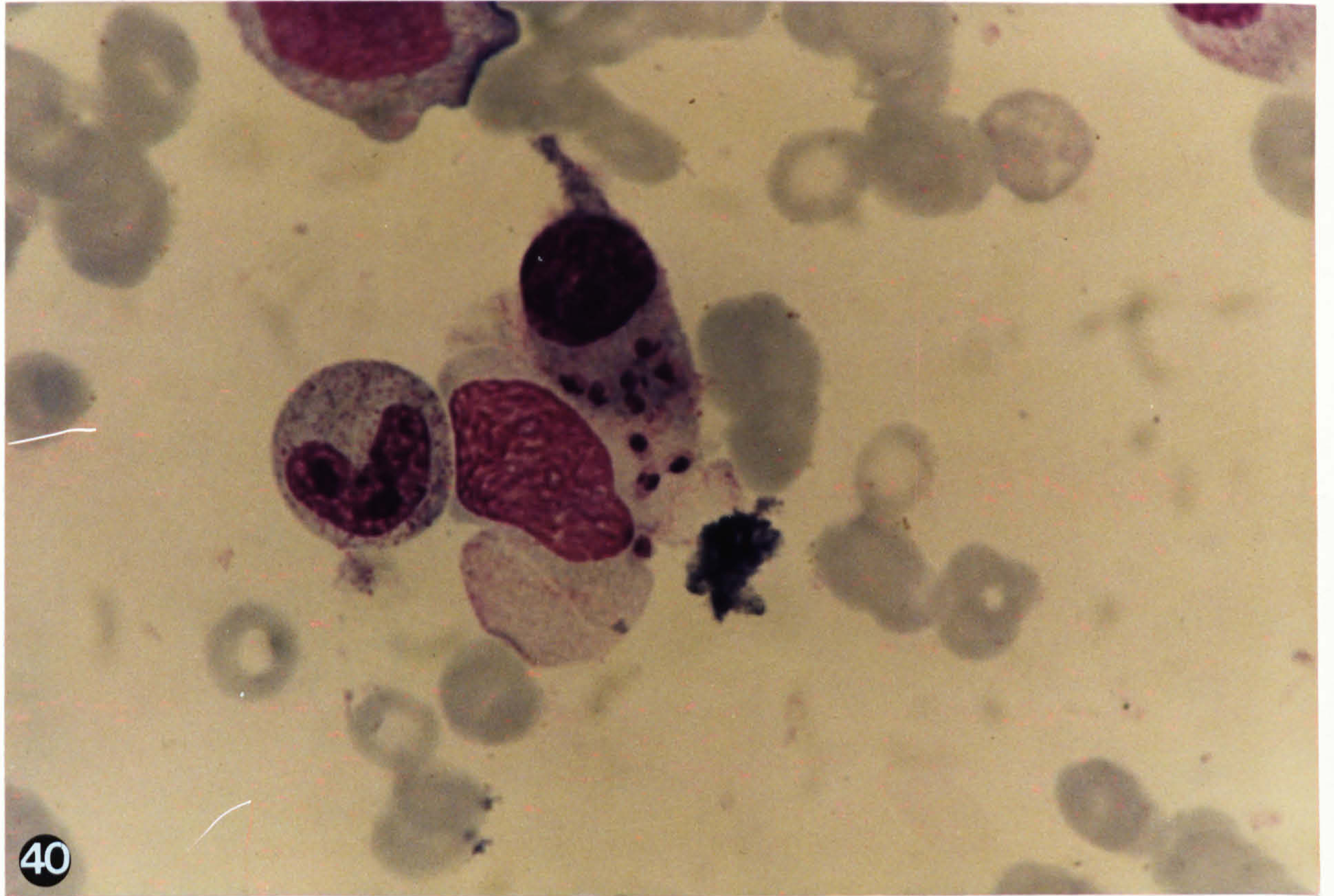
Impression smear of dog spleen

Plate 40. Amastigotes in a macrophage in the impression smear of spleen from dog No.15 from Mahayel. (This particular infection was cultured but the culture was lost before identification).


40

Amastigotes in tissue section of dog liver

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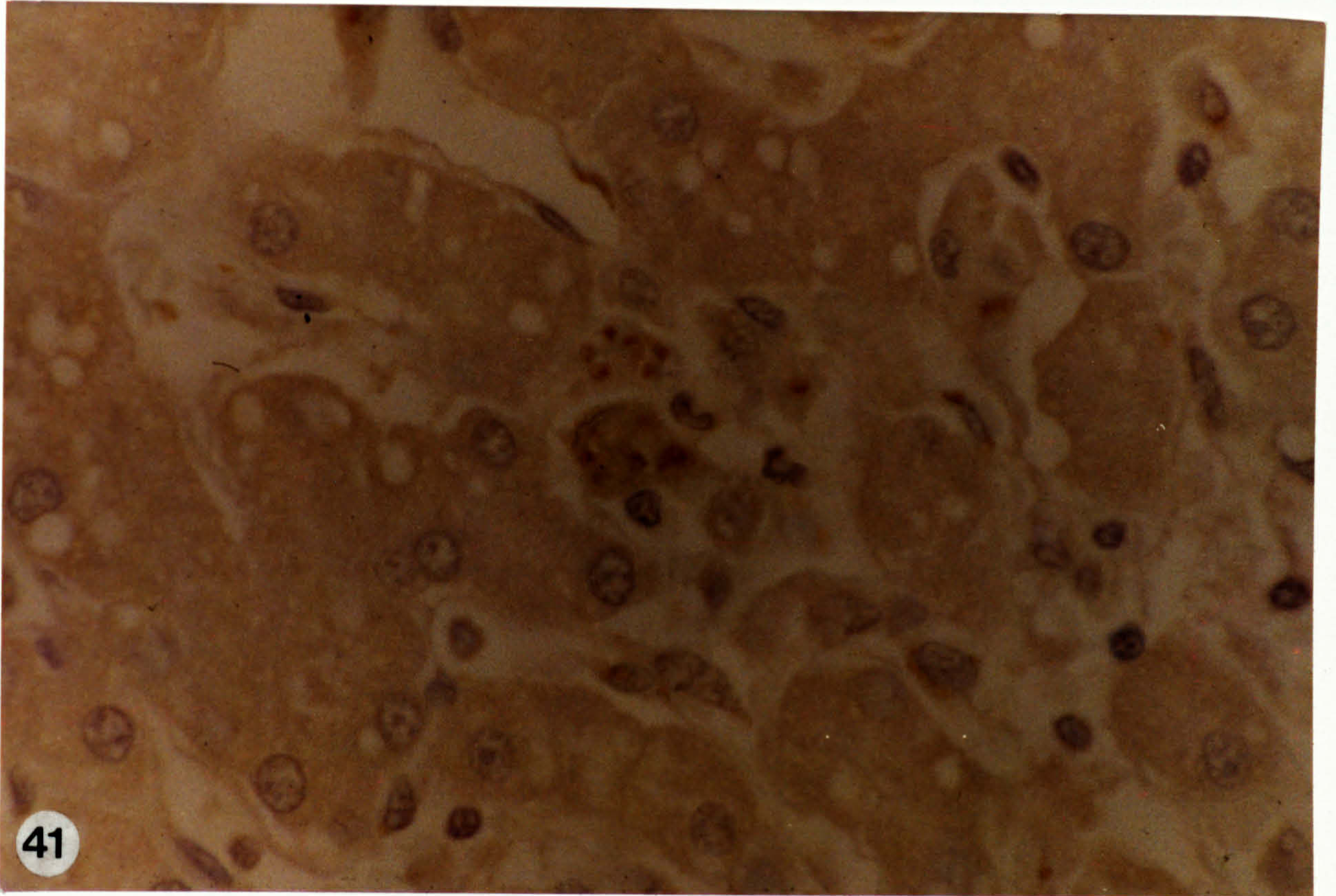
Plates 41 and 42. Amastigotes shown by immunoperoxidase staining of sections of liver from dog No. 81 from Al Khoba (X 1600). The parasites were cultured and identified as L.infantum zymodeme LON-49.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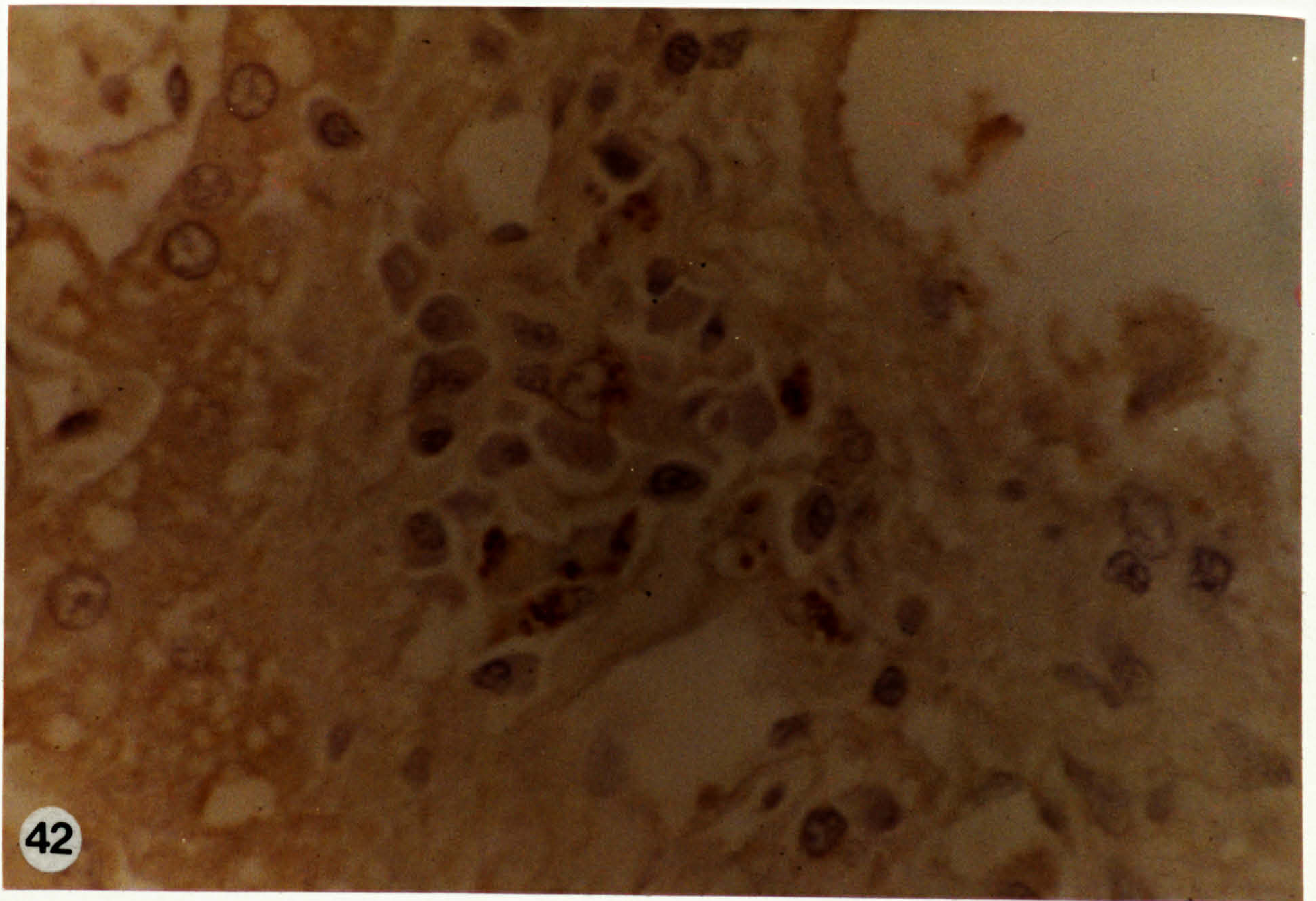

42

isolates from the same area which were L.donovani sensu lato LON-42 (see plate 36).

2.4 Serological examination

Seventeen blood spots from 88 dog blood spots were sero-positive by the ELISA technique and about 60% of these positives gave a higher titre than 1.0 optical density.

To increase the specificity, the cut-off point was set at 0.5 and any values above that were considered to be positive. The ELISA value of seropositive dogs with the parasitological and morphological results are shown in Table 28. The prevalence of visceral leishmaniasis among dogs in the study area and the ELISA results corresponding to other parasitological and morphological examinations data are shown in Tables 29 and 30 respectively.

3. Hyrax Study

While the main animal study was carried out in dogs, the possible role of other animals such as the foxes, wolves, cats, hyraxes was taken into account. Unfortunately the time available was insufficient to carry out all the investigations which would be needed for a better understanding of the epidemiology of visceral leishmaniasis. However, during the dog collections, one hyrax (plate 43) was shot and examined for VL and CL. Smears and cultures from nose, ear liver and spleen were negative. In fact, the hyrax is common in the mountainous area and the local people

TABLE 28 The seropositive ELISA values of dogs compared with parasitological and morphological results.

Dog	ELISA*	Parasitology**	Lesions	Heptomegaly	Splenomegaly	Remarks
D10	0.95	-	Ears eroded	-	-	-
D12	0.53	-	-	-	+	-
D15	1.00	+	-	-	-	-
D19	1.00	+	Ears eroded	-	+	Long nails
D20	1.10	+	-	-	+	-
D50	1.30	-	-	+	+	-
D54	1.30	-	-	-	-	-
D57	0.52	-	-	-	-	-
D61	1.32	-	-	-	-	-
D63	0.60	-	-	-	-	-
D68	1.14	-	-	-	-	-
D73	0.64	+	-	-	-	-
D76	1.40	+	Ear eroded	-	-	Long nails
D79	0.57	-	-	-	+	-
D80	1.20	-	-	+	+	-
D81	1.40	+	Skin ulcer	-	-	Lesions on thigh & lips
B83	0.55	-	-	-	+	-

*The cut-off point was an absorbence (optical density) value of 0.5; any value greater than 0.5 was considered to be positive. All the values were calculated using the formula:

Corrected optical density (OD) = (1/positive control value) x sample value

**Spleen and liver impression smears, tissue sections and cultures.

TABLE 29 Prevalence of canine leishmaniasis in southwest Saudi Arabia.

Diagnostic technique:	Bani-Thwa, Mahayel	Al Khoba, Gizan	Total
Parasitological examination			
No. of dogs examined	40	49	89
No. with parasites	3 (7.5%)	3 (6.1%)	6 (6.7%)
Serological examination			
No. of dogs examined	40	48	88
No. ELISA positive	5 (12.5%)	12 (24.5%)	17 (19.3%)
Leishmania isolates*	0	3	3

*Isoenzymes typical of *Leishmania infantum* LON-49.

TABLE 30 ELISA results corresponding to other parasitological and morphological data from Bani-Thwa, Mahayel and Al Khoba, Gizan.

Description:	ELISA positive	ELISA negative	Total
Parasitologically positive	6	0	6
Parasitologically negative	11	71	82
With ear and/or skin lesions	4	3	7
With heptomegaly	2	4	6
With splenomegaly	7	13	20

254

Hyrax species

255

Plate 43. A hyrax caught in the Mahayel foothills.
No parasites were found in this animal.

43

hunt it as a source of food. The hyrax shown was caught in the Mahayel foothills area at an altitude of approximately 1500 m above sea level.

4. Result of the Direct Agglutination Test

Blood spots from 69 children and 19 dogs were tested for antibodies against L.donovani sensu lato by using both the ELISA technique and DAT.

Of the blood spots from children, 5 out of 69 were positive by the DAT, whereas by the ELISA technique, 13 out of 69 were positive. Of the dog blood spots, 5 out of 19 were DAT positive, whereas by the ELISA technique, 8 out of 19 were positive.

The results of the DAT compared with those of the ELISA on human bloods are shown in Table 31, and of dog bloods in Table 32. It is seen that two bloods from children were DAT positive but ELISA negative, even though the overall positive rate of the ELISA (18.8%) was much higher than that of the DAT (7.2%). In the dog samples all the DAT positive samples were also ELISA positive, but, as in the human blood samples, the DAT positivity rate (26.3%) was lower than that of the ELISA positive rate (42.1%).

The DAT positive reciprocals of the end titre dilutions and the ELISA seropositive values of both child and dog bloods are shown in Figures 24 and 25 respectively (DAT microtitre plate loaded with dog samples is shown in plate 44).

TABLE 31 The results of DAT and ELISA tests in the children's blood spot survey for visceral leishmaniasis

		ELISA		
		Positive	Negative	Total
DAT	Positive	3	2	5
	Negative	10	54	64
	Total	13	56	69

TABLE 32 The results of DAT and ELISA tests in the dog blood spot survey for visceral leishmaniasis

		ELISA		
		Positive	Negative	Total
DAT	Positive	5	0	5
	Negative	3	11	14
	Total	8	11	19

Fig. 24. Comparison of the results of DAT and ELISA tests on child blood spots from southwest Saudi Arabia (n = 69)

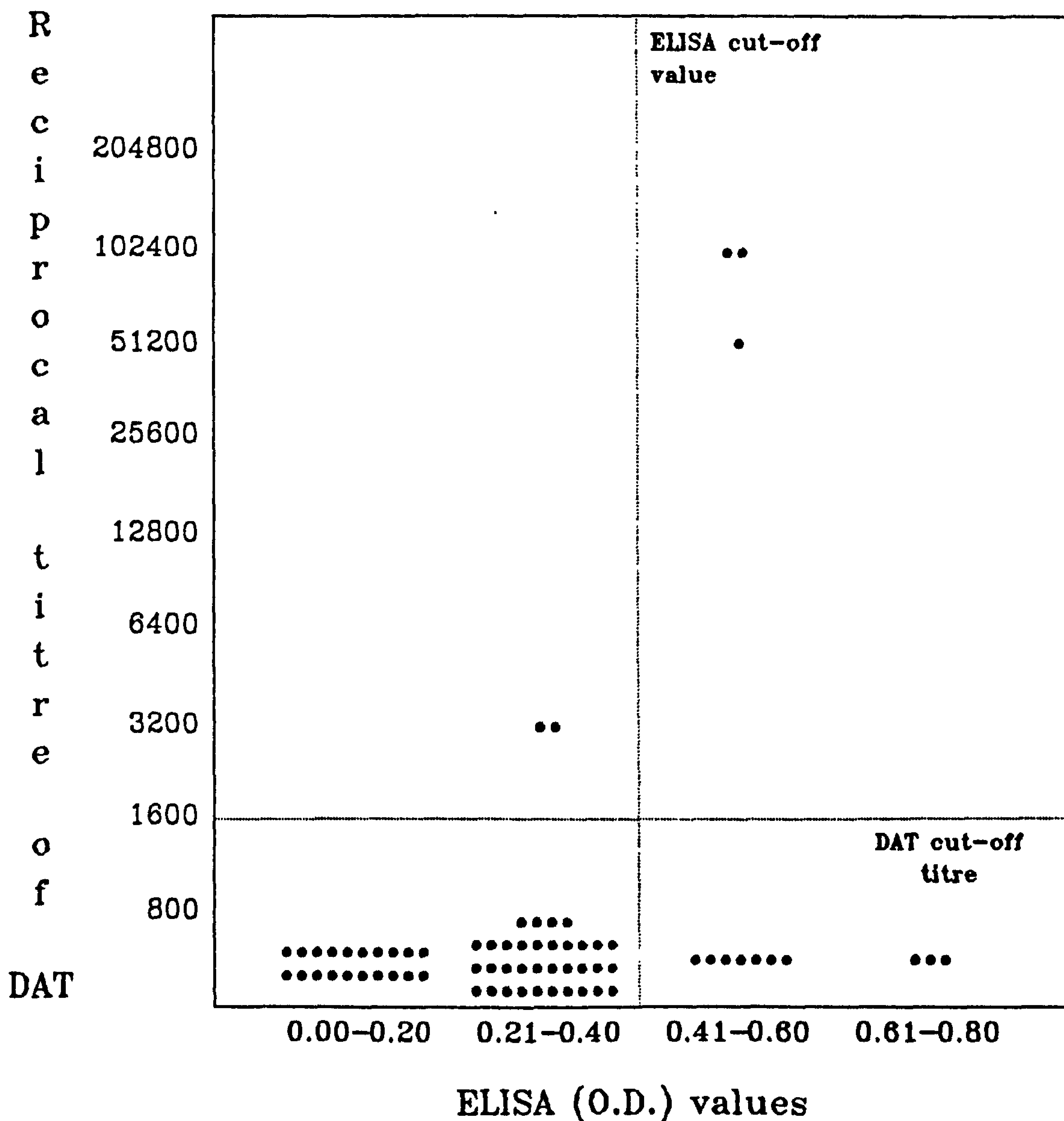
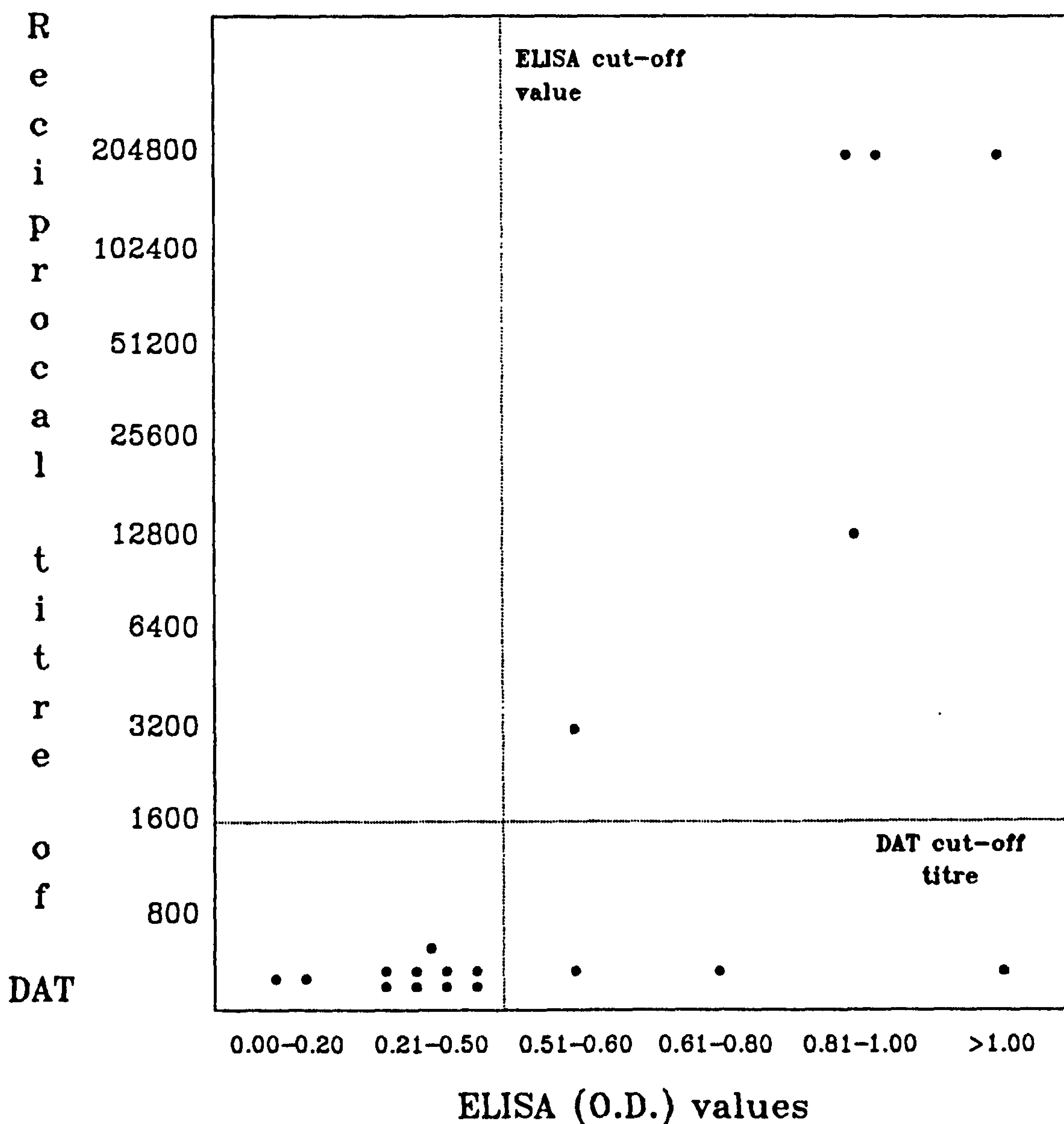


Fig. 25. Comparison of the results of DAT and ELISA tests on dog blood spots from southwest Saudi Arabia (n = 19)



Microtitre plate for Direct Agglutination Test (DAT)

Plate 44. A microtitre plate with V-shaped wells used for the DAT on blood spot samples from dogs

Dilution

Row 1 A - H - negative control

Row A 2 - 12 - positive control

Doubling dilutions Nos. 2 - 12 to 1 : 204,800

Samples B2 - H2

Dog D20 + to 1 : 204,800

D19 + to 1 : 102,400

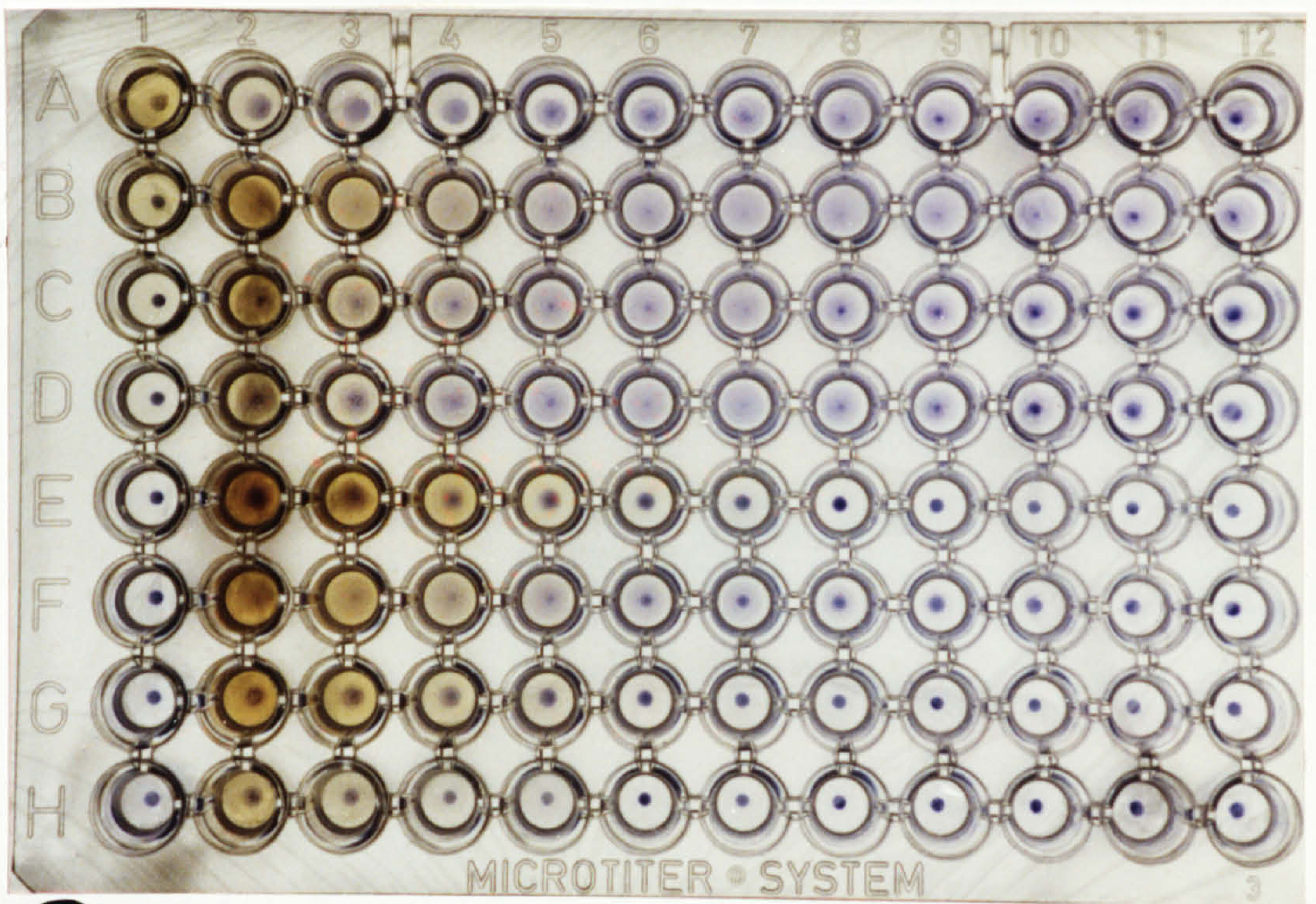
D15 + to 1 : 204,800

D12 + to 1 : 3200

D10 + to 1 : 102,400

D83 + to - : 1600

D82 Negative



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Part 4. Entomological Studies

1. Faunal Composition and Geographical Distribution

A total of 8914 sandflies were collected from the fixed stations, night catches, surveys in the vicinities of VL patients, and additional random collections. The numbers of specimens of Phlebotomus species collected by each activity are given in Table 33.

The collection sites were in different areas, see Map No. 8, Chapter II, and covered both highlands and lowlands reaching to the end of the eastern side of the Asir plateau near Bishah and Tathlith. The coastal areas were represented in the collection by Al-Khoba and Haroob in the Gizan province.

Sandfly classification to species level was carried out in specimens of the genus Phlebotomus. Flies belonging to the genus Sergentomyia were not classified for practical reasons. The species of the genus Phlebotomus which were found in the collecting sites in the study area were:

(i) Ph.sergenti Parrot, 1917
(ii) Ph.bergeroti Parrot, 1934
(iii) Ph.arabicus Theodor, 1953
(iv) Ph.alexandri Sinton, 1928
(v) Ph.orientalis Parrot, 1936
(vi) Ph.papatasi Scopoli, 1786

Ph.sergenti and Ph.bergeroti were collected from all the areas, both in the high and lowlands. Ph.arabicus were

TABLE 33 The numbers of sandflies of the Genera Phlebotomus and Sergentomyia captured in southwest Saudi Arabia (December 1986–December 1987).

Species	Sex	Fixed stations Highland	Fixed stations Lowland	Kala-azar survey	Night Catches	Other	Total	Grand Total
Genus Phlebotomus								
sergenti	M	388	151	6	0	31	576	1116
	F	297	139	15	61	28	540	
bergeroti	M	82	972	118	5	312	1489	3547
	F	53	1412	77	344	172	2058	
arabicus	M	194	0	3	0	35	232	288
	F	44	0	0	0	12	56	
alexandri	M	6	39	6	0	0	51	122
	F	4	50	9	8	0	71	
orientalis	M	62	9	0	0	7	78	107
	F	24	4	0	0	1	29	
papatasi	M	0	21	4	0	39	64	93
	F	0	6	1	19	3	29	
Genus Sergentomyia	M	75	1170	216	0	159	1620	3641
	F	70	1525	278	0	148	2021	
Total:		1299	5498	733	437	947		8914

collected from the highlands only. Ph.orientalis were mainly collected in the highlands, and in contrast to this, Ph.alexandri was collected from the lowlands, especially in the foothills.

Ph.papatasi was found to be very rare in the fixed stations and the majority of this collection was from Bishah and Tethlith which are situated in the Asir plateau; its ecotype is considered to be oases in the desert area.

2. Relative Abundance of Species

2.1 In the highlands

Figure 26 shows the percentage of each Phlebotomus species in the Asir highland plateau collections. It is clear that the dominant species was Ph.sergenti, 59%, followed by Ph.arabicus, 21%,Ph.bergeroti, 12%, Ph.orientalis,7% and Ph.alexandri 1% respectively. No Ph.papatasi were collected from this area.

2.2 In the lowlands

Figure 27 shows that the Ph.bergeroti is the dominant species found in the lowlands, 85%. Ph.sergenti was found to be the next dominant species in this area, 10% and Ph. alexandri 3%. There were very few Ph.orientalis or Ph. papatasi in this area, 1%, n = 40 of both species. The Phlebotomus species collected from the kala-azar patient surveys and night catches are present at the same order of abundance as these species collected from the fixed stations in the lowlands. As mentioned in Chapter II, section 5.4,

Fig. 26. Proportions of five *Phlebotomus* species in the total phlebotomine catches (Highlands, light traps and sticky traps, indoors and outdoors, December 1986 – December 1987)

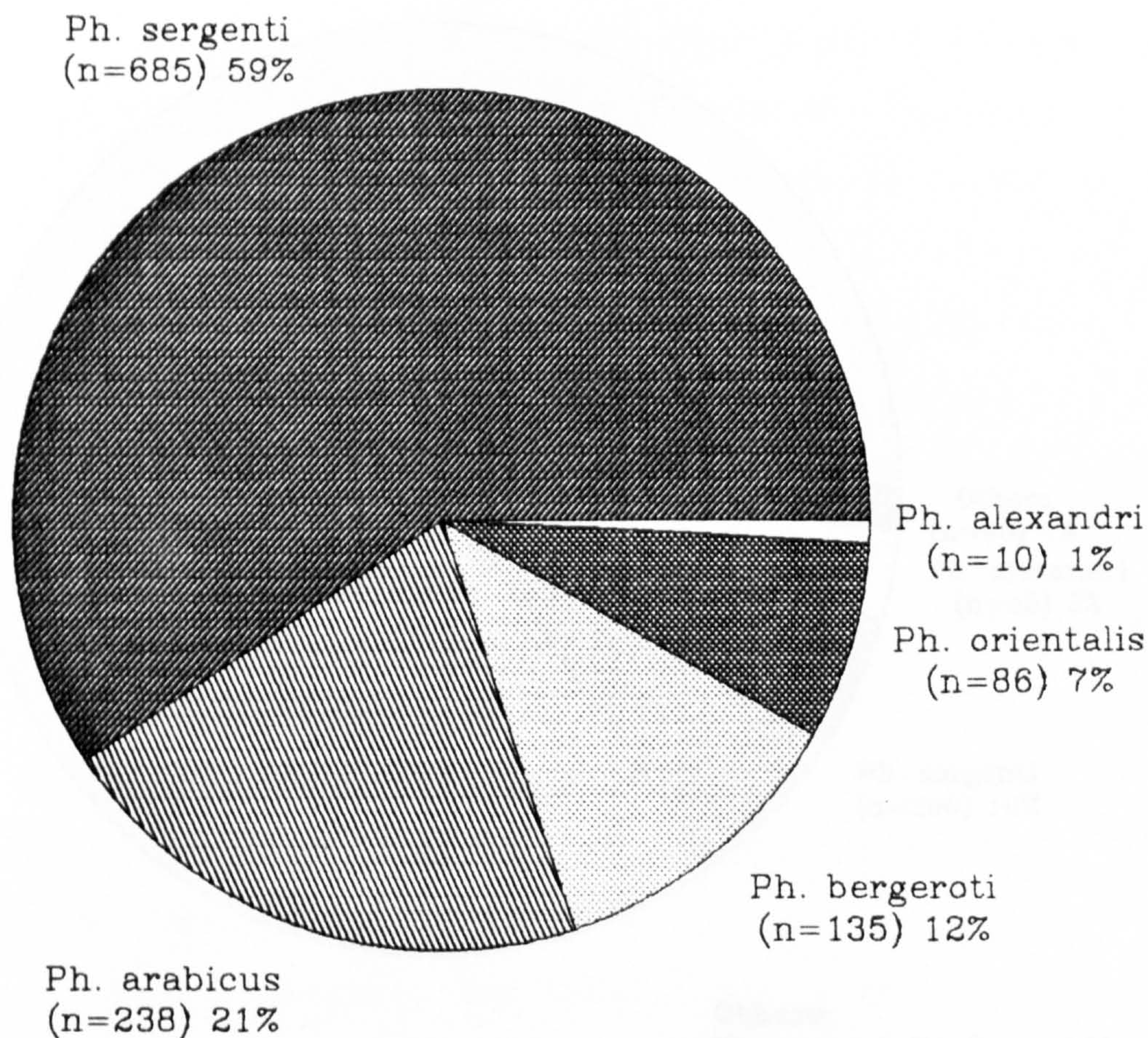
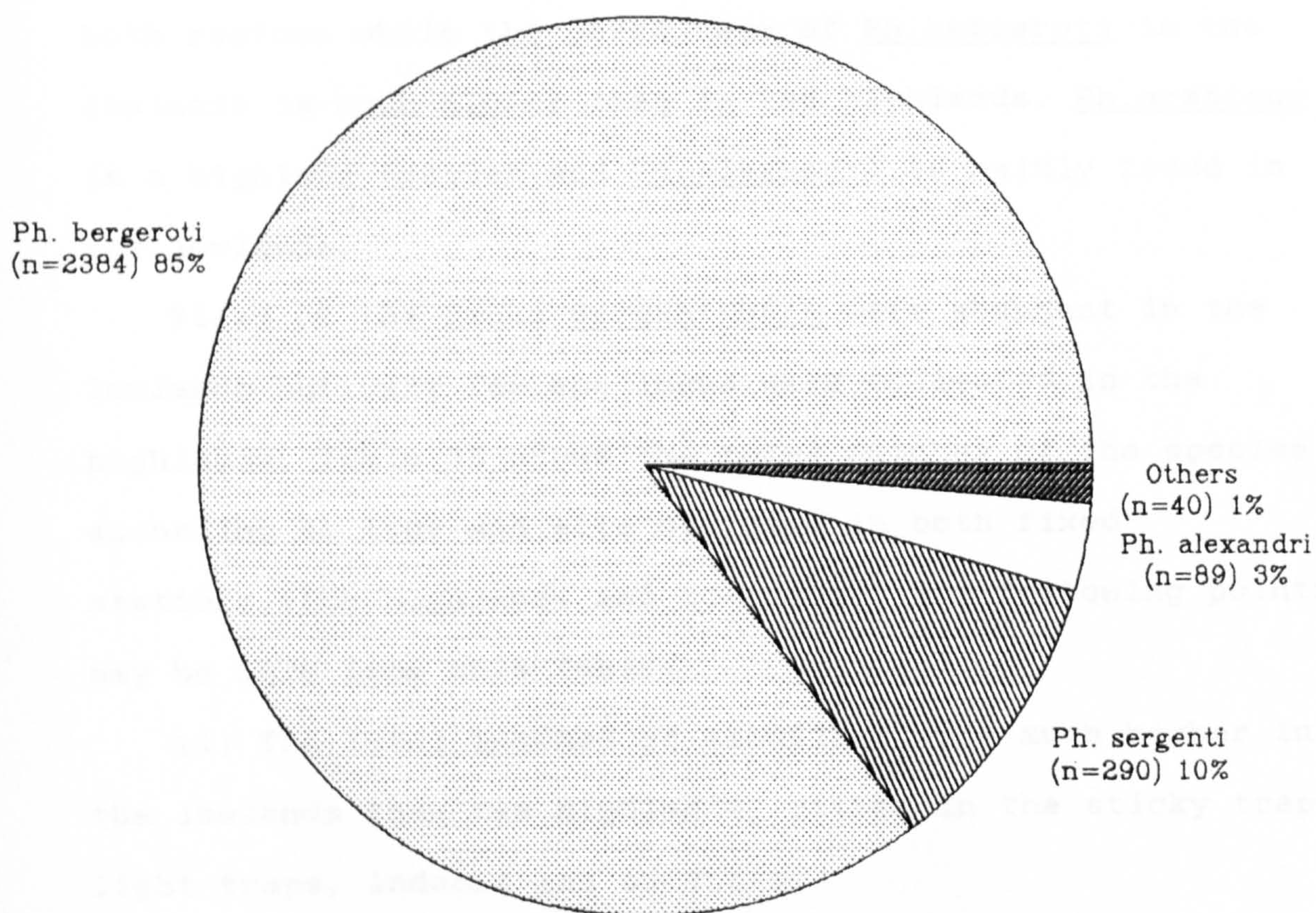


Fig. 27. Proportions of *Phlebotomus* species in the total phlebotomine catches (Lowlands, light traps and sticky traps, indoors and outdoors, December 1986 - December 1987)



Others:
Ph. orientalis (n = 13)
Ph. papatasi (n = 27)

these activities were carried out mainly in the lowlands, and only two kala-azar patients were investigated in the highlands (Khamis Mushat and Khaber Al-Gnoob). Details of the collections from the night catches and the kala-azar patient surveys are given in Tables 34 and 35.

Figure 28 compares the relative abundance of each species in the highlands and lowlands collected from the fixed stations. It is seen that Ph.sergenti is abundant in both regions while the percentage of Ph.bergeroti in the lowlands is much higher than in the highlands. Ph.arabicus is a highland species and Ph.alexandri is mainly found in the lowlands.

Flies of the genus Sergentomyia were abundant in the lowlands but very few specimens were collected in the highlands. Table 36 shows the total catches of the species according to type and site of traps in both fixed stations,(the highlands and lowlands). The following points may be made from this Table:

(i) The total catches of sandflies were much higher in the lowlands than the highlands, either in the sticky traps, light traps, indoors and outdoors.

(ii) In both regions, the total catches by light traps were significantly higher than the total catches by sticky traps ($X^2 = 38.2$, 1df, $p < 0.01$), except that Sergentomyia species were significantly more frequent in sticky traps than in the light traps ($X^2 = 43.1$, 1df, $p < 0.01$).

(iii) Except the Ph.sergenti in the highlands, all the

TABLE 34 Night catches of sandflies

Date	Village	Altitude (m)	*Phlebotomus* species: *bergeroti* M	*bergeroti* F	Other species:	M	F
08/11/86	Khat	475	0	25	*papatasi*	0	1
15/11/86	Bigrah	400	0	6	-	-	-
03/12/86	Khatba	540	5	0	*sergenti*	0	12
					alexandri	0	1
15/12/86	Khatba	540	0	2	*sergenti*	0	33
15/12/86	Bigrah	400	0	14	-	-	-
20/01/87	Khat	475	0	0	-	-	-
27/01/87	Khat	475	0	6	-	-	-
02/02/87	Bani-Thwa	650	0	8	-	-	-
02/02/87	Bani-Thwa	650	0	8	-	-	-
10/02/87	Bani-Thwa	650	0	4	-	-	-
30/03/87	Bani-Thwa	650	0	2	-	-	-
22/06/87	Bani-Thwa	650	0	2	-	-	-
09/06/87	Khat	475	0	1	*papatasi*	0	2
					sergenti	0	2
24/03/87	Mahayel	480	0	32	-	-	-
16/03/87	Bigrah	400	0	10	-	-	-
14/03/87	Khatba	540	0	3	-	-	-
21/04/87	Khatba	540	0	9	-	-	-
21/06/87	Khatba	540	0	9	-	-	-
06/04/87	Khat	475	0	20	-	-	-
07/07/87	Khat	475	0	5	-	-	-
15/08/87	Khat	475	0	18	-	-	-
09/09/87	Khat	475	0	2	-	-	-
18/08/87	Bigrah	400	0	14	-	-	-
09/09/87	Bigrah	400	0	8	-	-	-
24/08/87	Khatba	540	0	30	-	-	-
08/09/87	Khatba	540	0	6	-	-	-
25/08/87	Bani-Thwa	650	0	6	-	-	-
15/09/87	Quz	350	0	4	-	-	-
N.A.	Al-Fatyeha	165	0	9	*papatasi*	0	4
N.A.	Wadi Itwad	170	0	24	*papatasi*	0	2
10/02/87	Wadi Hasswa	750	0	3	*alexandri*	0	7
					sergenti	0	5
09/02/87	Al-Jof	680	0	2	*sergenti*	0	2
10/02/87	Harub	475	0	0	*papatasi*	0	6
					sergenti	0	1
08/03/87	Khobah	175	0	52	*papatasi*	0	4
					sergenti	0	6

TABLE 35 Sandfly species collected from the homes of Kala-azar patients, using light traps and sticky traps.

Date	Locality	Altitude (m)	*Phlebotomus* species: *bergeroti* M	F	Other species:	M	F	*Sergentomyia* species M	F
09/02/87	Al-Jof	680	11	7	-	-	-	8	20
01/03/87	Kamismutar	550	5	7	-	-	-	74	117
16/03/87	Bani-Thwa	650	5	3	*sergenti*	0	2	2	14
17/03/87	Thaluth Al-Manzar	460	1	4	*papatasi* *sergenti*	1 1	0 1	0	0
13/04/87	Al-Rash	500	1	2	-	-	-	6	6
14/06/87	Khat	475	6	7	*sergenti*	1	1	24	17
15/06/87	Farshat	440	6	6	*alexandri*	1	0	11	9
15/06/87	Bani-Thwa	650	2	7	*alexandri*	1	0	8	20
29/06/87	Bani-Thwa	650	1	1	-	-	-	1	3
15/08/87	Mahayel	480	11	5	-	-	-	13	10
22/08/87	Ratmah	860	29	2	*sergenti* *alexandri*	4 0	8 1	7	8
30/08/87	Khabar Al-Jnoob	1700	26	9	*papatasi* *arabicus* *sergenti*	3 3 0	1 0 1	22	4
06/09/87	Khatba	540	0	1	-	-	-	14	9
06/09/87	Thaluth Amarah	420	0	2	-	-	-	6	15
06/09/87	Mahayel	480	7	1	-	-	-	9	17
08/11/87	Khamis Mushayt	1950	2	1	*alexandri*	0	2	3	5
08/11/87	Al-Farsha	730	5	5	*alexandri*	4	6	8	4

Fig. 28. Relative proportions of *Phlebotomus* spp. at the Highland and Lowland stations

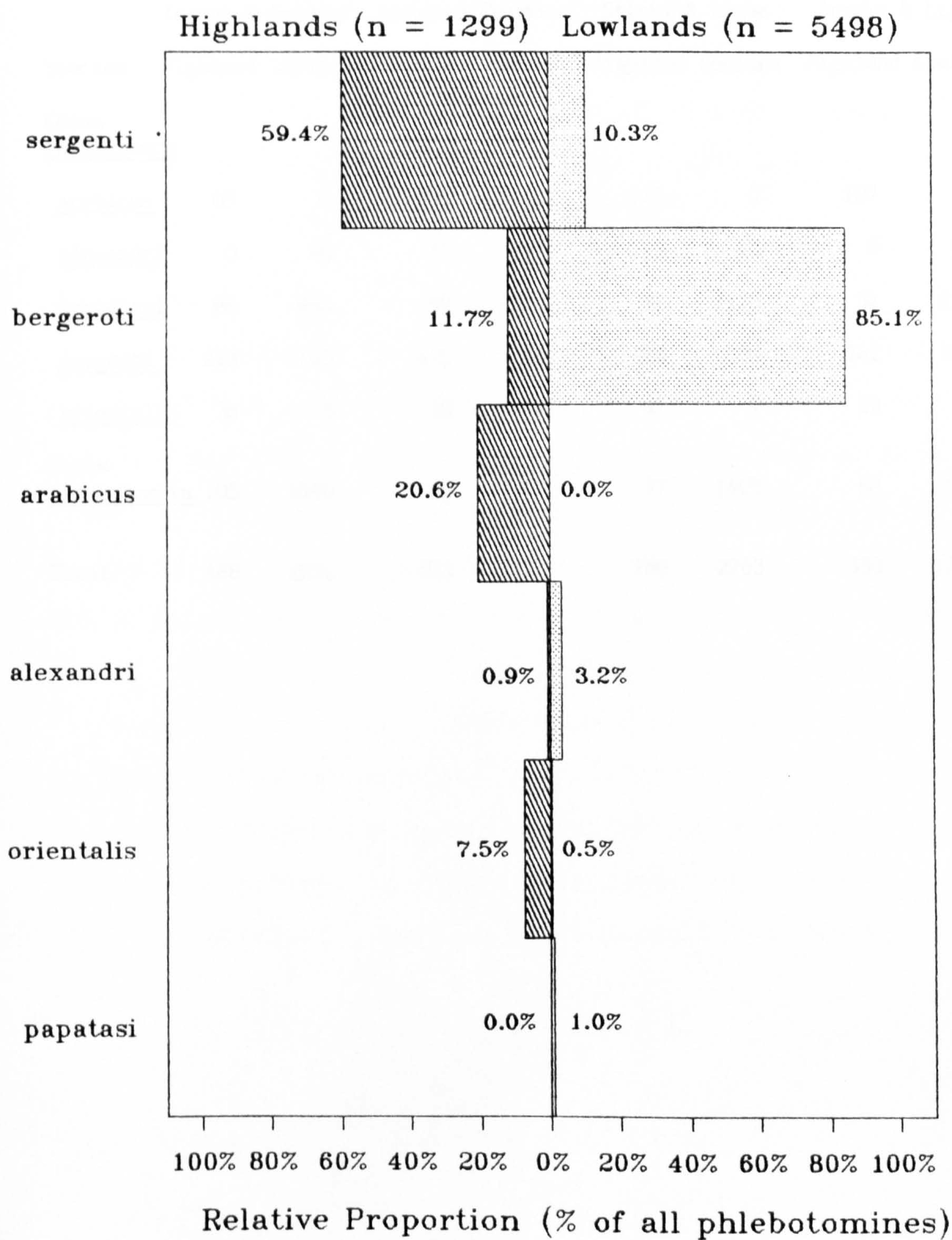


TABLE 36 Total number of sandflies collected at the fixed stations according to trap type and location.

	According to trap type				According to trap location			
	Sticky traps Indoor & Outdoor		Light traps Indoor & Outdoor		Indoor Sticky & Light		Outdoor Sticky & Light	
Species	Highland	Lowland	Highland	Lowland	Highland	Lowland	Highland	Lowland
Genus *Phlebotomus*								
arabicus	68	0	170	0	131	0	107	0
alexandri	0	48	10	41	10	43	0	46
bergeroti	66	964	69	1420	81	1128	54	1256
sergenti	213	145	472	145	423	180	262	110
orientalis	36	5	50	8	47	7	39	6
Genus *Sergentomyia*	105	1490	40	1205	77	1405	68	1290
Total:	488	2652	811	2819	769	2763	530	2708

other *Phlebotomus* species in both regions show no significant differences in outdoor and indoor catches. *Sergentomyia* spp. also were significantly commoner indoors than outdoors (X^2 = 5.2, 1df, $p < 0.02$).

(iv) As mentioned, the *Ph.sergenti* in the highlands showed significant differences between indoor and outdoor catches, which may have been related to the heavy rainfall in August. (X^2 = 50.246, 1df, $p < 0.01$). This and other factors will be discussed in Chapter VI.

3. *Sex Ratio*

The accumulated data collected from the entomological fixed stations indicated that the sex ratio was different from one species to another and affected by type and site of traps, in addition to the season, eg, *Ph.sergenti* and *Ph. bergeroti* in the lowlands and highlands, see Figures 29, 30 and 31 which show less than one male per female in winter with a slight increase in late summer to 2 males per female.

Some species of males were much higher, such as *Ph. arabicus* with a sex ratio of 4 males per female, see Figures 32, and 33. Figure 34 shows the proportion numbers of male *Phlebotomus* species at Shuhat (highlands) and indicates a slightly greater number of males indoors than outdoors.

Fig. 29. Seasonal sex ratio of *Ph. sergenti* at Shuhat (Highlands), (Light traps and sticky traps, indoors and outdoors)

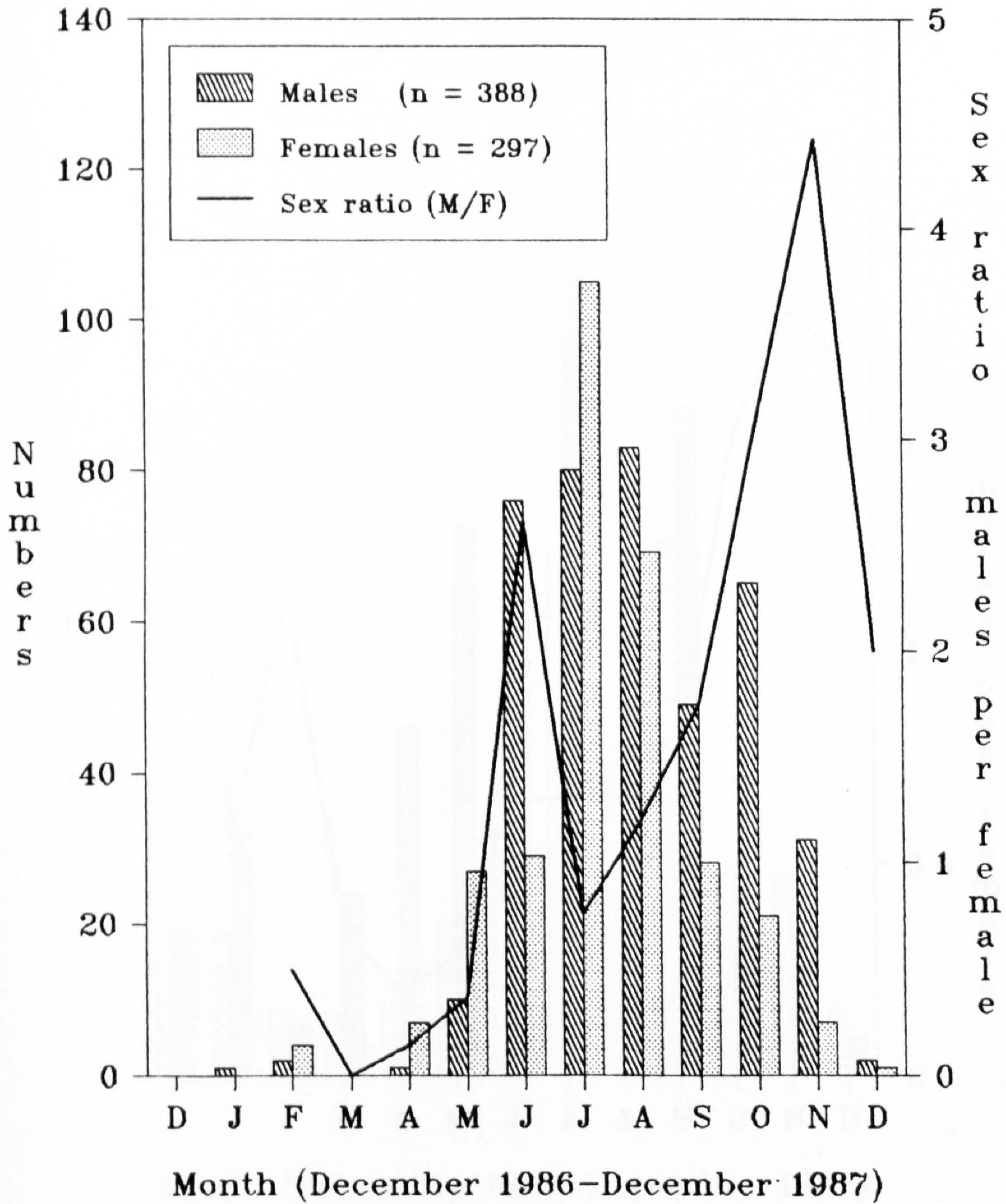


Fig. 30. Seasonal sex ratio of *Ph. bergeroti* at Bani–Thwa (Lowlands), (light traps and sticky traps, indoors and outdoors)

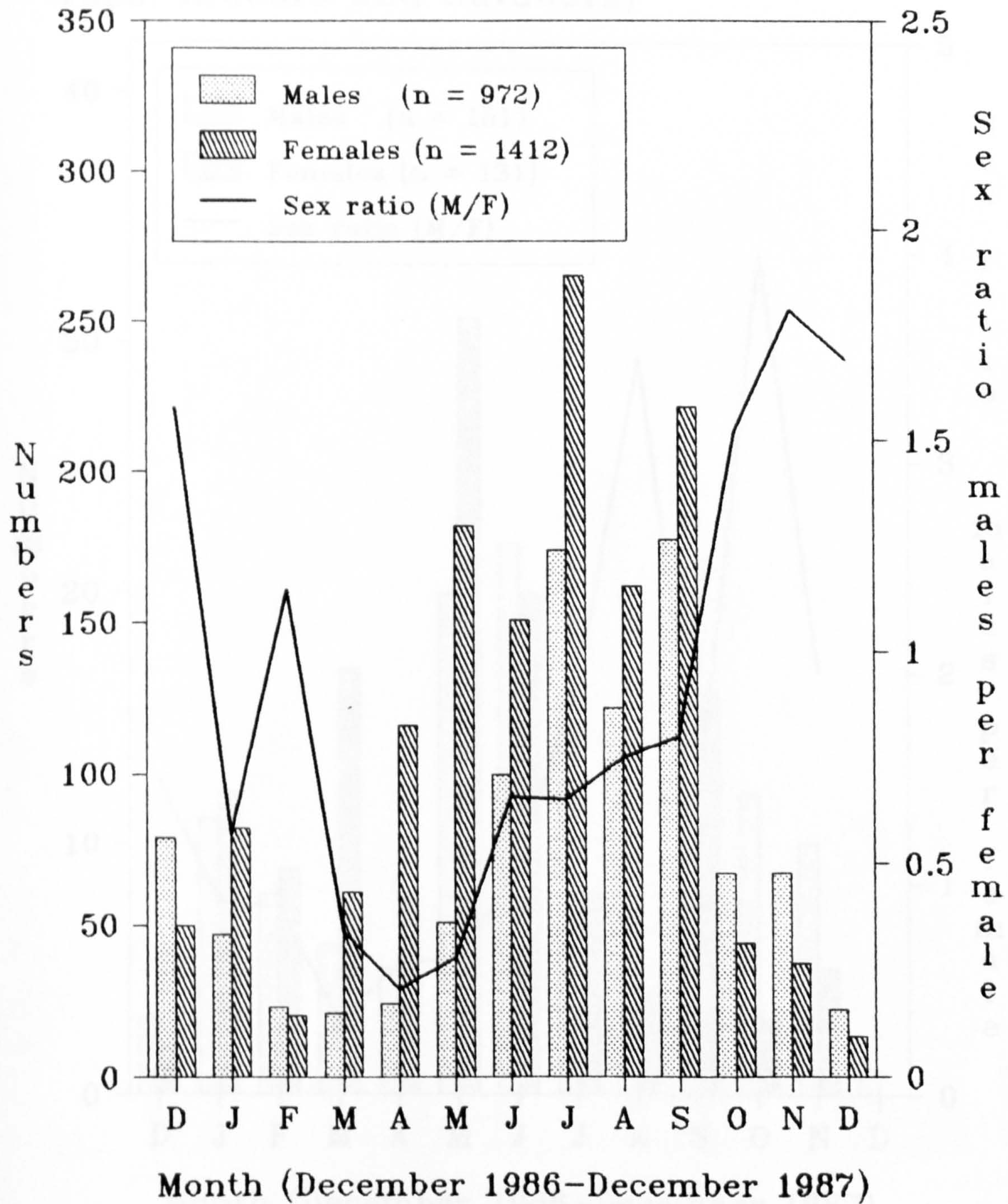


Fig. 31. Seasonal sex ratio of *Ph. sergenti* at Bani–Thwa (Lowlands), (light traps and sticky traps, indoors and outdoors)

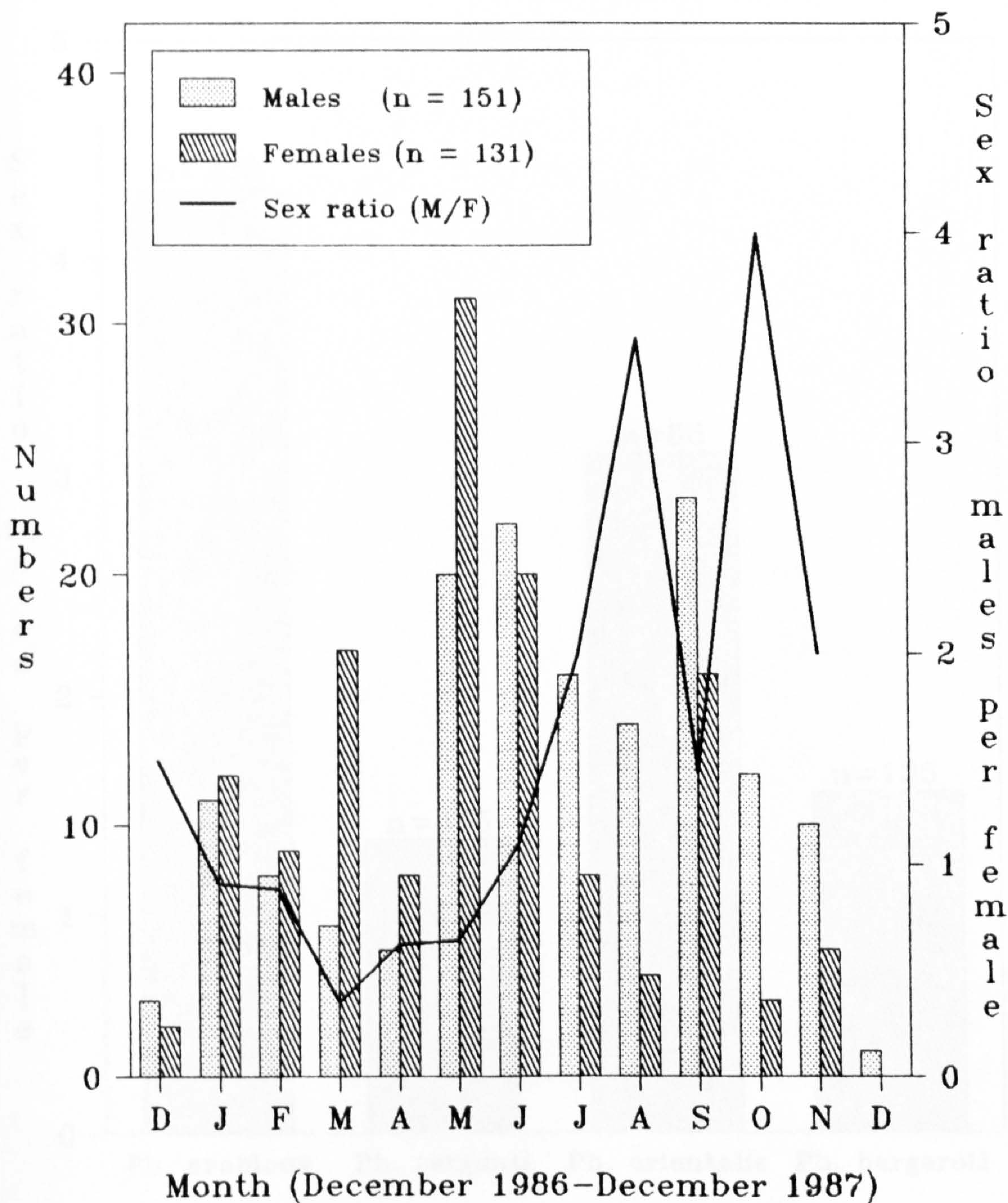


Fig. 32. Sex ratio of *Ph. arabicus*, *Ph. sergenti*, *Ph. orientalis* and *Ph. bergeroti* at Shuhat (Highlands)

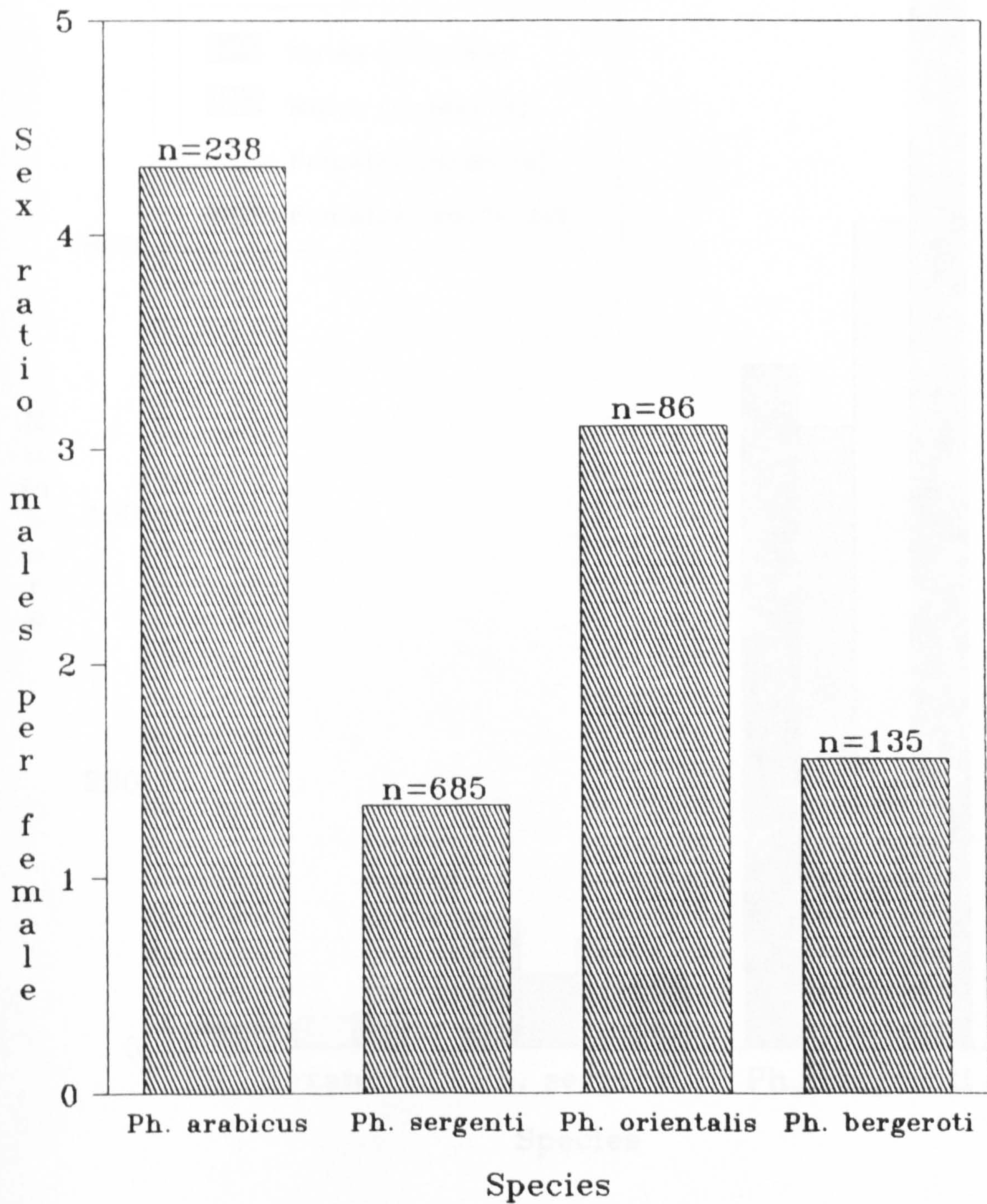


Fig. 33. Numbers of *Ph. alexandri*, *Ph. sergenti* and *Ph. bergeroti* (lowlands), (December 1986 - December 1987)

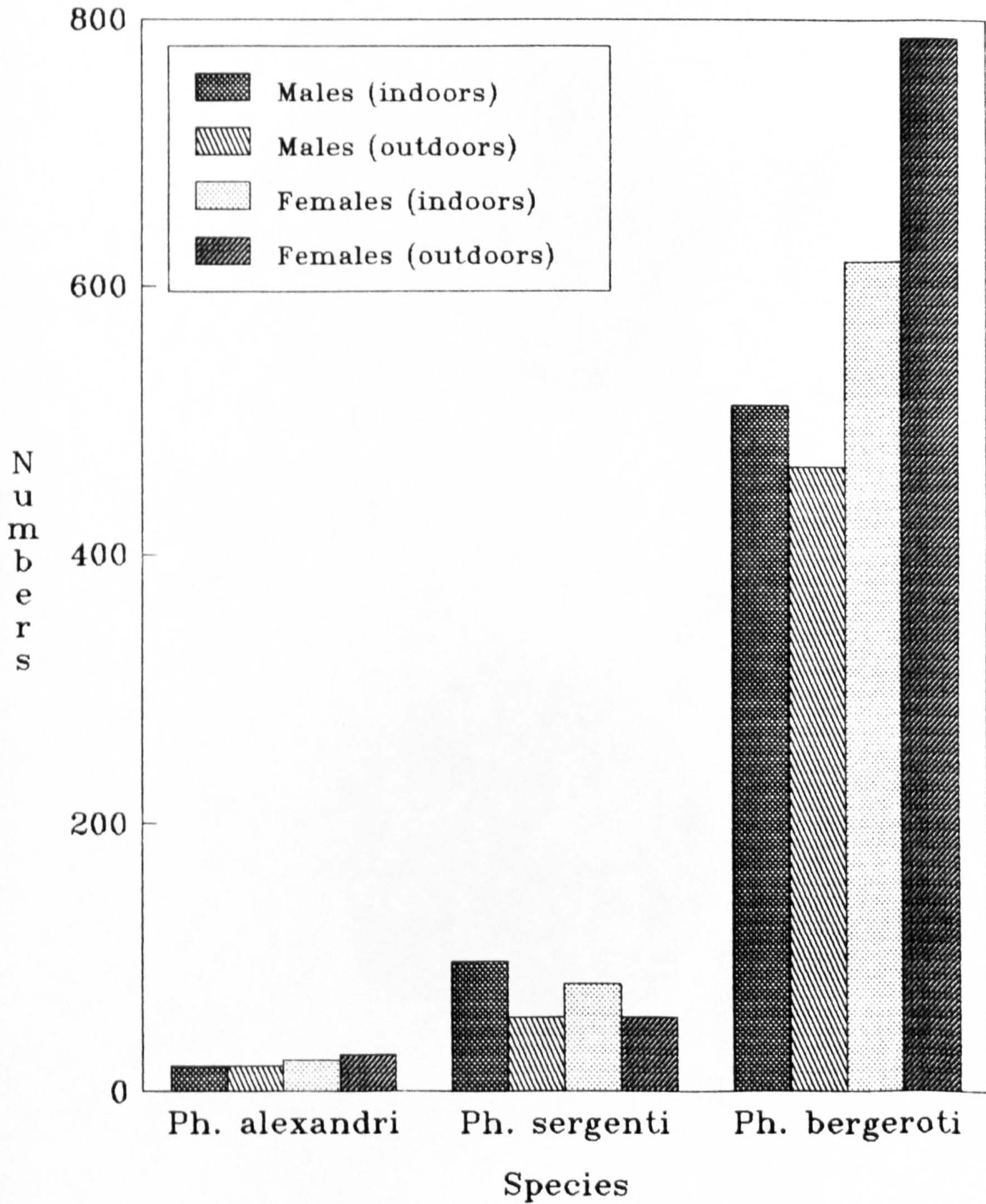
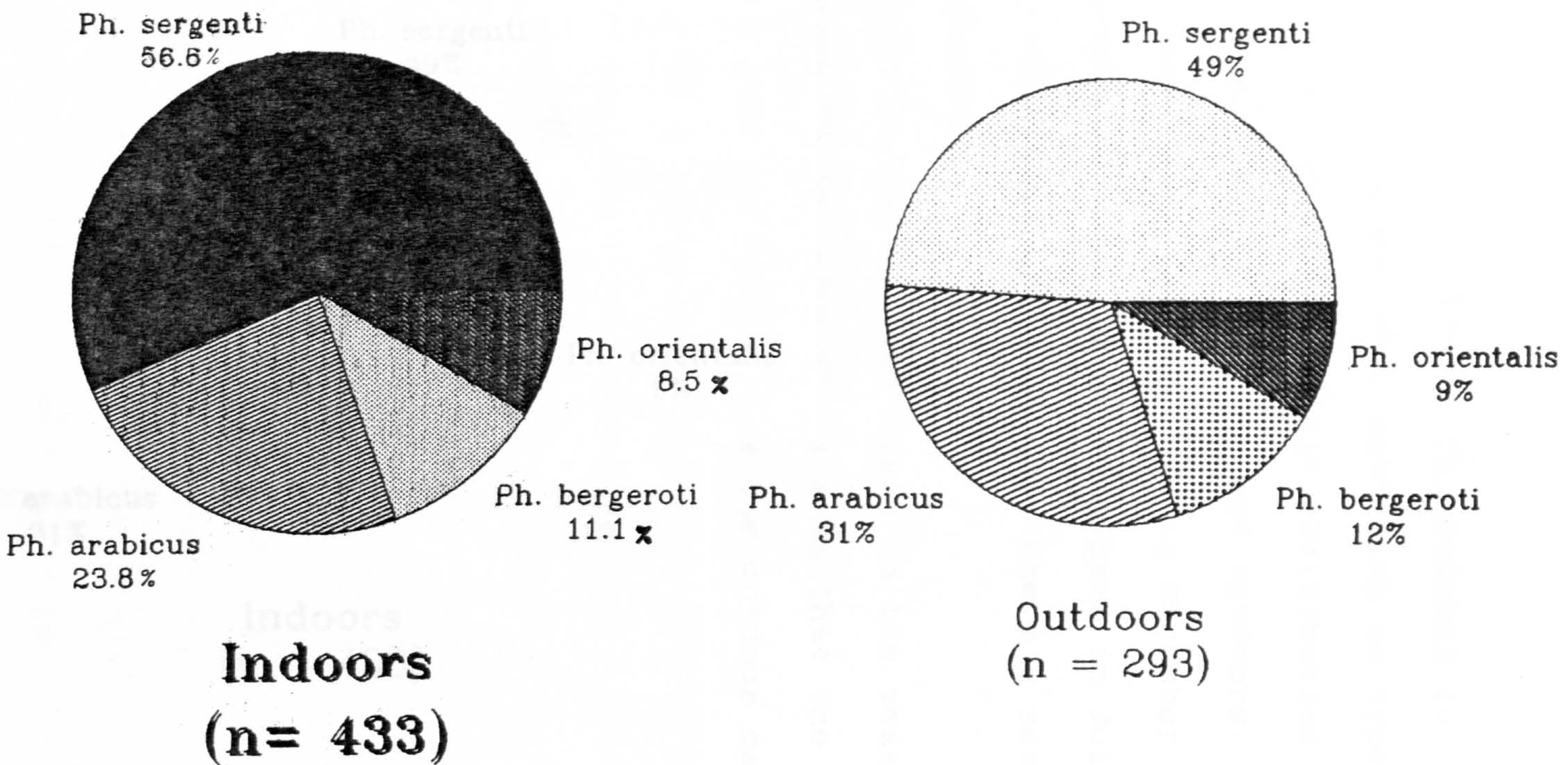


Fig. 34. Proportions of four *Phlebotomus* species males in the total phlebotomine catches at Shuhat (Highlands), (light traps and sticky traps)



4. Seasonal Variations in Sandfly Populations

4.1 The highlands

(i) Ph.sergenti

The monthly distribution of *Ph.sergenti* in the highlands is presented in different ways according to type and site of traps. Figure 35 shows the monthly distribution of the total catches (male and female) indoors and outdoors. The *Ph. sergenti* population was nearly zero in December 1986 and increased from April 1987 to reach a peak in July, then subsequently decreased until October where a second, smaller peak was observed.

The effect of the site of traps on the seasonality is shown in Figure 36 in which it is seen that the indoor catches had a peak in July, while the outdoor catches had a tendency to peak a month later in August. The peak in July of the indoor collection was significantly higher than the peak of the outdoor collection in August ($X^2 = 53.06$, 1df, $p < 0.01$).

Epidemiologically the female population are of more concern than the males, and, for this reason, the data of the monthly distribution of females was presented separately, see Figure 37. This shows the pattern of *Ph. sergenti* female seasonality which was similar to that in Figure 36, and confirms the second late peak in August of outdoor female catches. This was significantly different from the peak of the indoor female peak in July ($X^2 = 10.4$, 1df, $p < 0.01$).

The collections from sticky traps were significantly less than those from the light traps (X^2 = 98, 1df, $p < 0.01$) which appeared to give constant fluctuations in seasonal distribution compared to light trap collections, see Figures 38 and 39.

(ii) Other _Phlebotomus_ species

The seasonal variations of other species in the highlands are presented in Figures 33, 34 and 35, for species of _Ph.bergeroti_, _Ph.orientalis_ and _Ph.arabicus_ respectively. The peaks of these species were in July, the same as _Ph. sergenti_ but, as mentioned before, the relative abundance of these species is much less than that of _Ph.sergenti_ and the male proportion was greater than the female, see Figure 32.

4.2 The lowlands

In the lowlands where _Ph.bergeroti_ is the dominant species, the population of this species shows two peaks, the first in July and the second in September (Figure 43). The total outdoor catches were significantly different from indoor catches (X^2 = 6.8, 1df, $p < 0.001$),(Figure 44). However, the pattern of monthly distribution was similar to that of the total catches in Figure 43 with two peaks in July and September. If we look at the situation from another angle and see the effect of the seasonality by types and sites of traps, we can see other pictures, eg, _Ph.bergeroti_ catches by light traps (indoor and outdoor) (Figure 45) show

Fig. 35. Seasonal distribution of *Ph. sergenti* at Shuhat (Highlands), (light traps and sticky traps, indoors and outdoors, sexes combined)

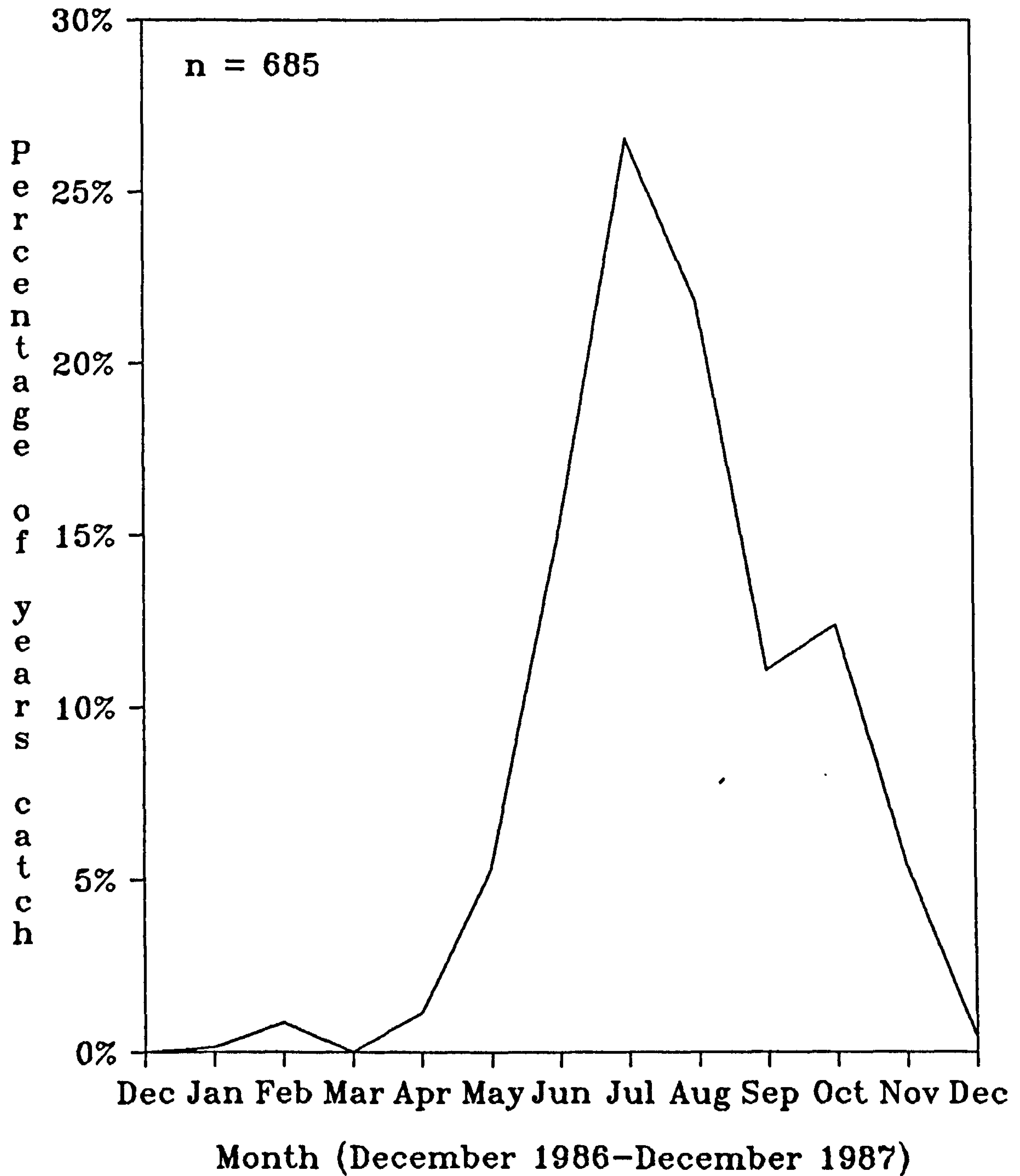
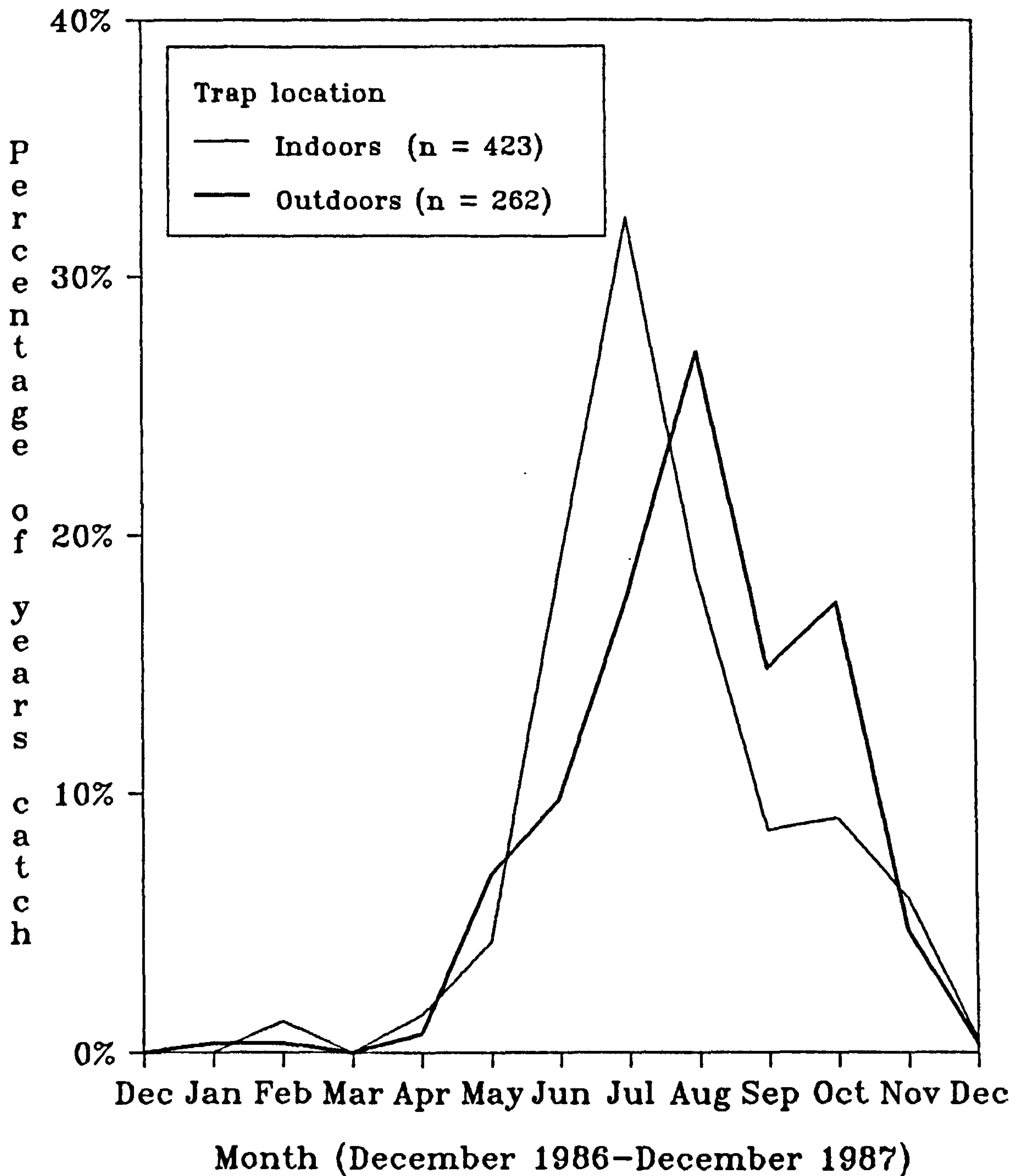


Fig. 36. Seasonal distribution of *Ph. sergenti* at Shuhat (Highlands) according to trap location (light traps and sticky traps, sexes combined)



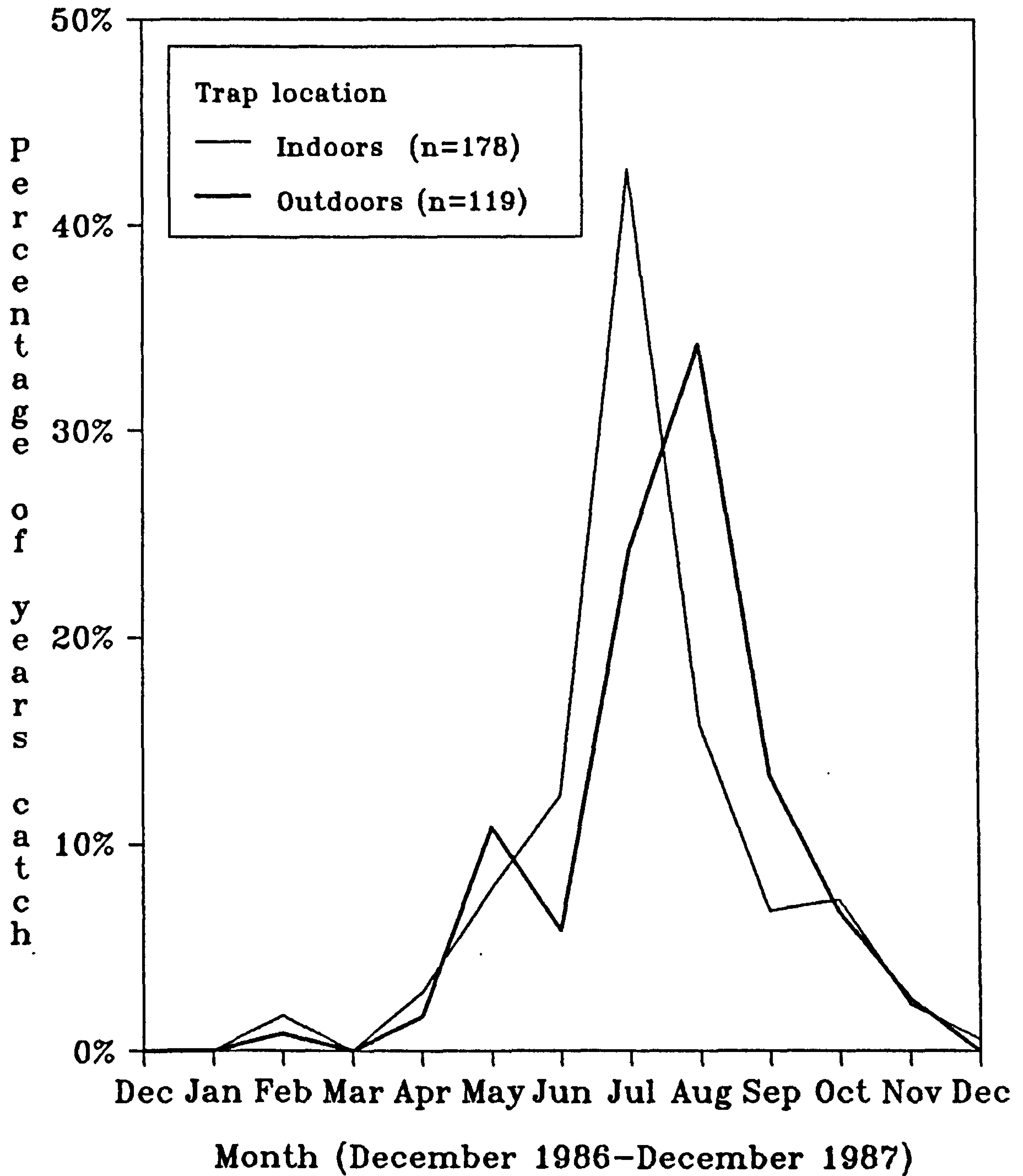


Fig. 37. Seasonal distribution of *Ph. sergenti* females at Shuhat (Highlands) according to trap location (light traps and sticky traps)

Fig. 38. Seasonal distribution of *Ph. sergenti* at Shuhat (Highlands) according to trap location (light traps, sexes combined)

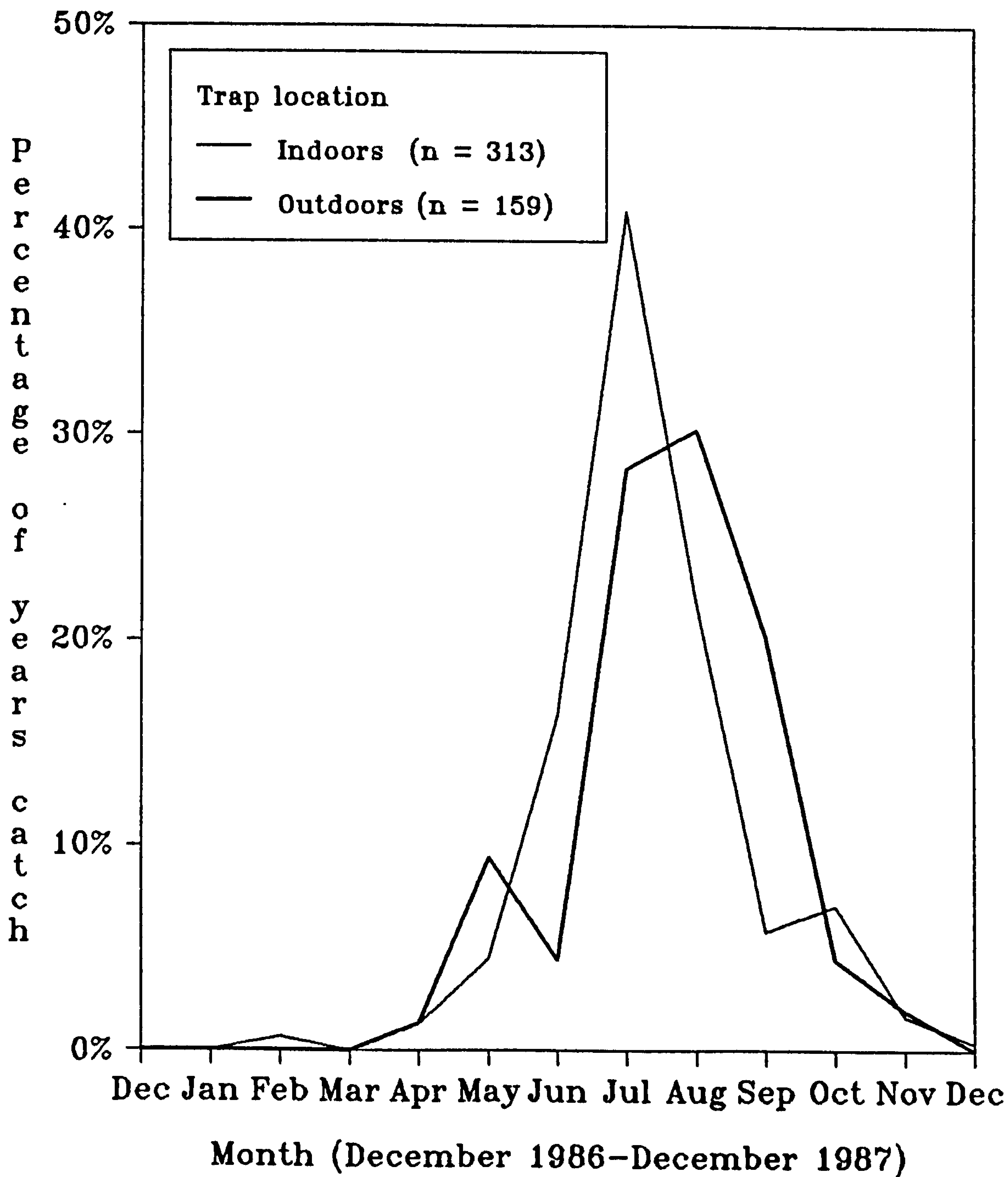


Fig. 39. Seasonal distribution of *Ph. sergenti* at Shuhat (Highlands) according to trap location (sticky traps, sexes combined)

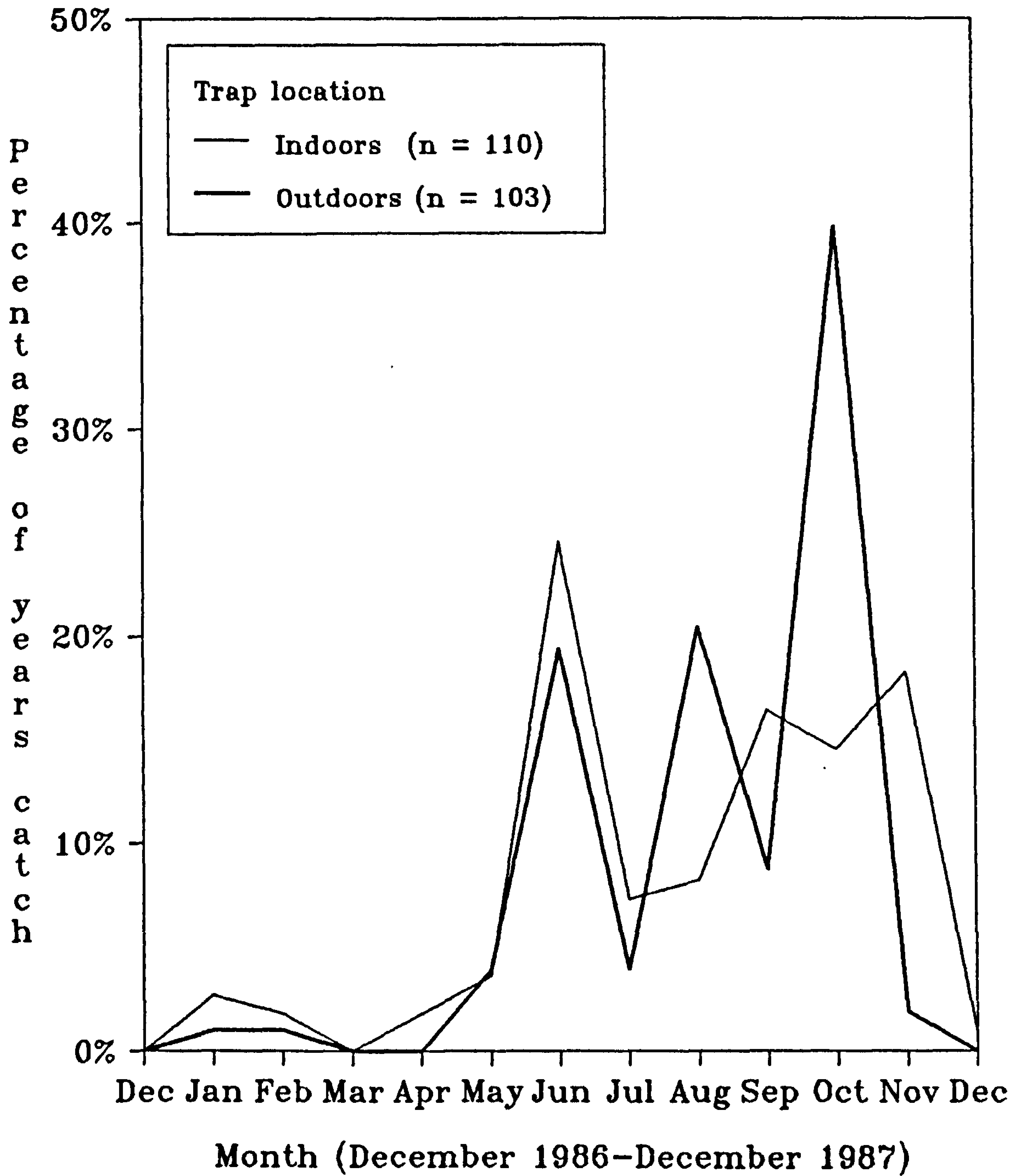


Fig. 40. Seasonal distribution of *Ph. bergeroti* at Shuhat (Highlands), (light traps and sticky traps, sexes combined)

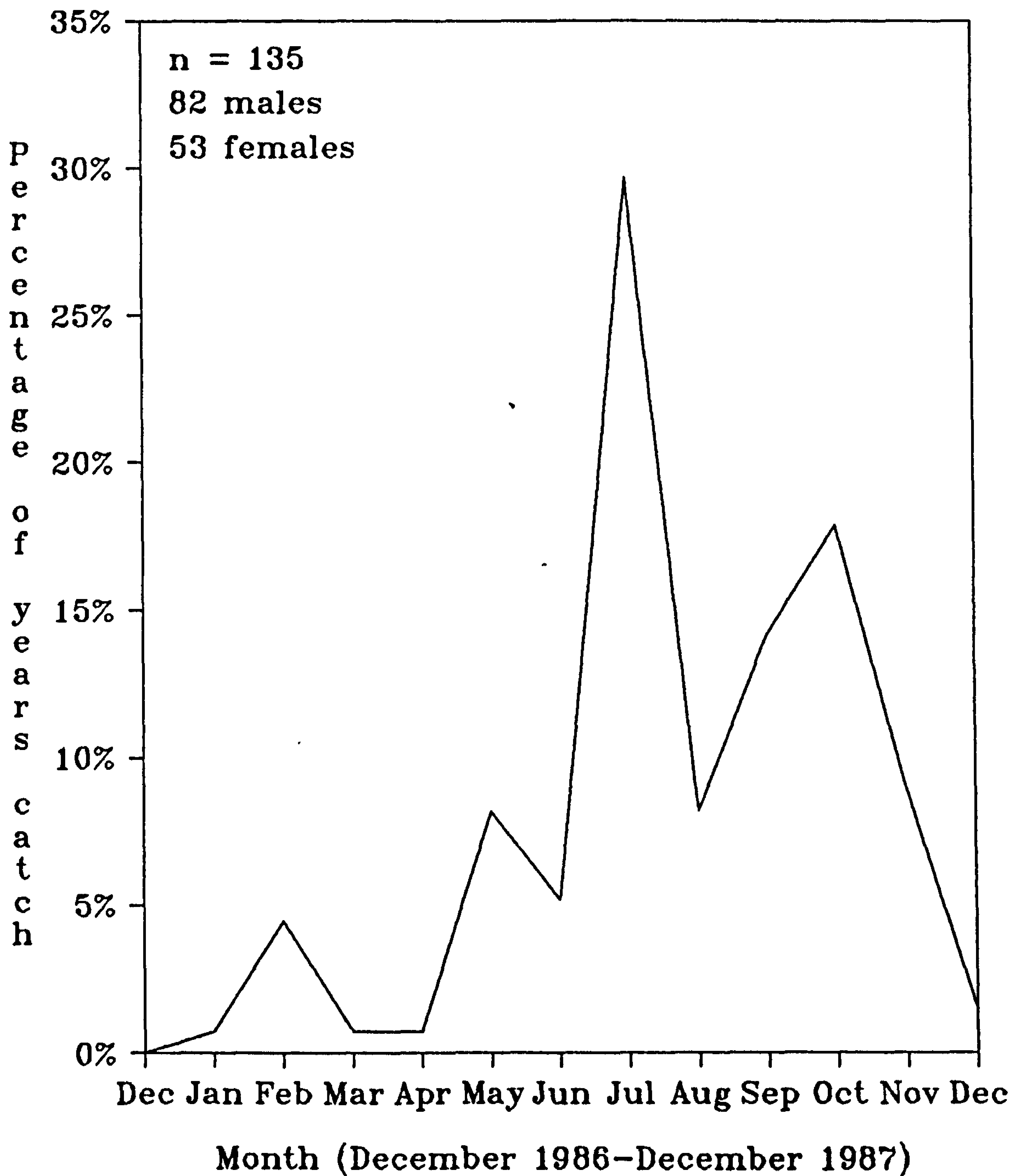


Fig. 41. Seasonal distribution of *Ph. orientalis* at Shuhat (Highlands), (light traps and sticky traps, sexes combined)

Fig. 42. Seasonal distribution of *Ph. arabicus* at Shuhat (Highlands), (light traps and sticky traps, sexes combined)

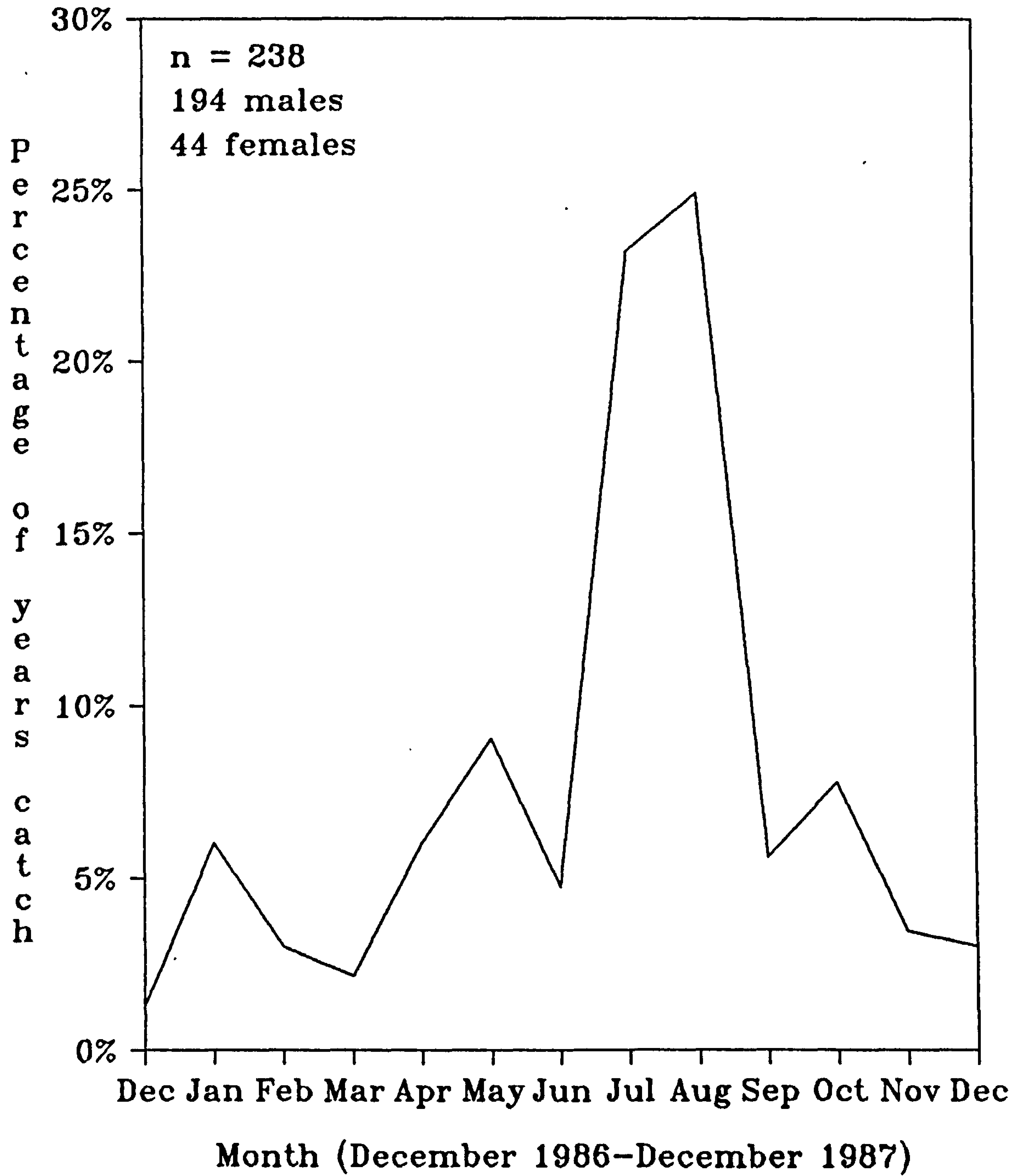


Fig. 43. Seasonal distribution of *Ph. bergeroti* at Bani–Thwa (Lowlands), (light traps and sticky traps, sexes combined)

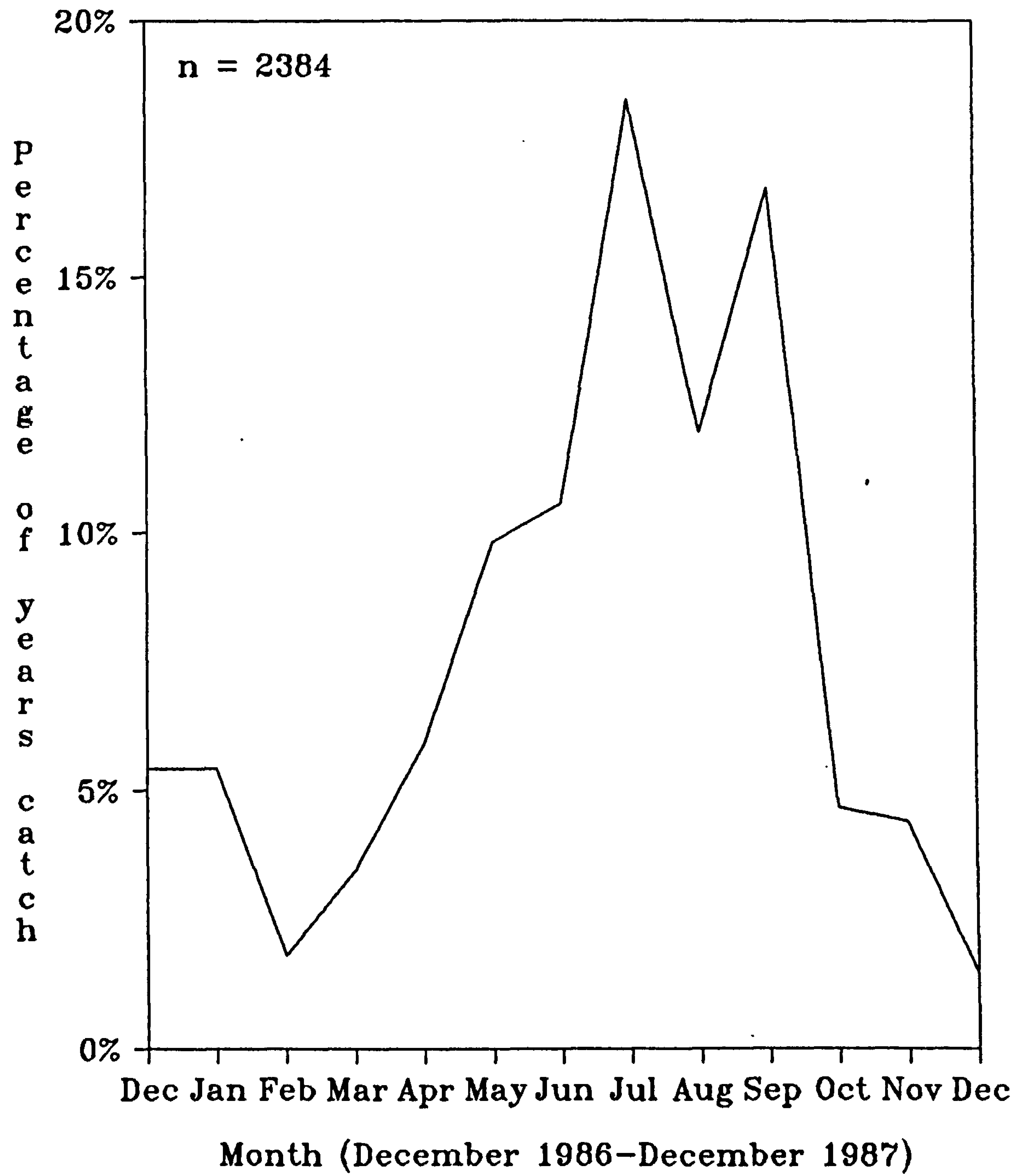


Fig. 44. Seasonal distribution of *Ph. bergeroti* at Bani–Thwa (Lowlands) according to trap location (light traps and sticky traps, sexes combined)

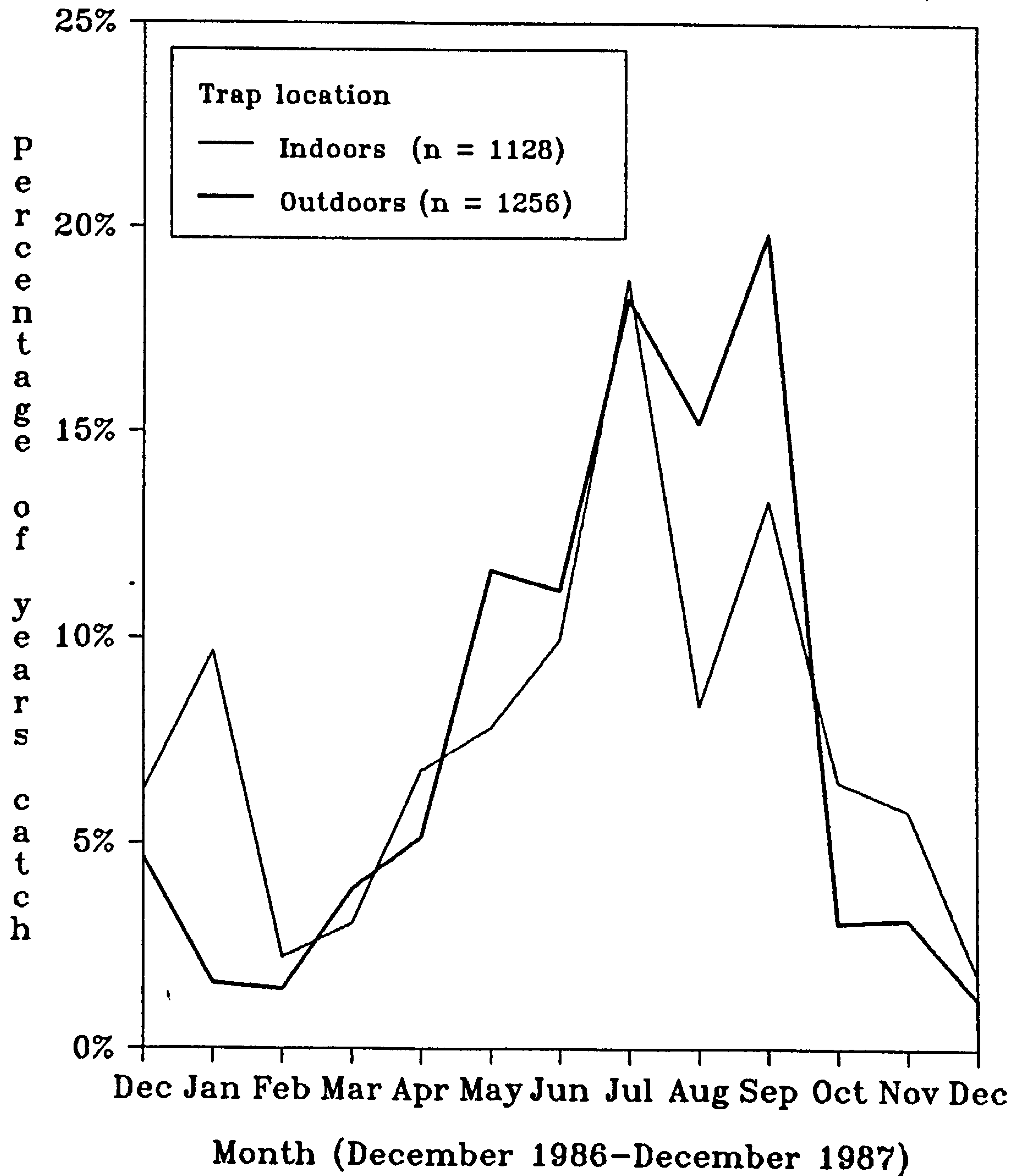


Fig. 45. Seasonal distribution of *Ph. bergeroti* at Bani–Thwa (Lowlands), (light traps, sexes combined)

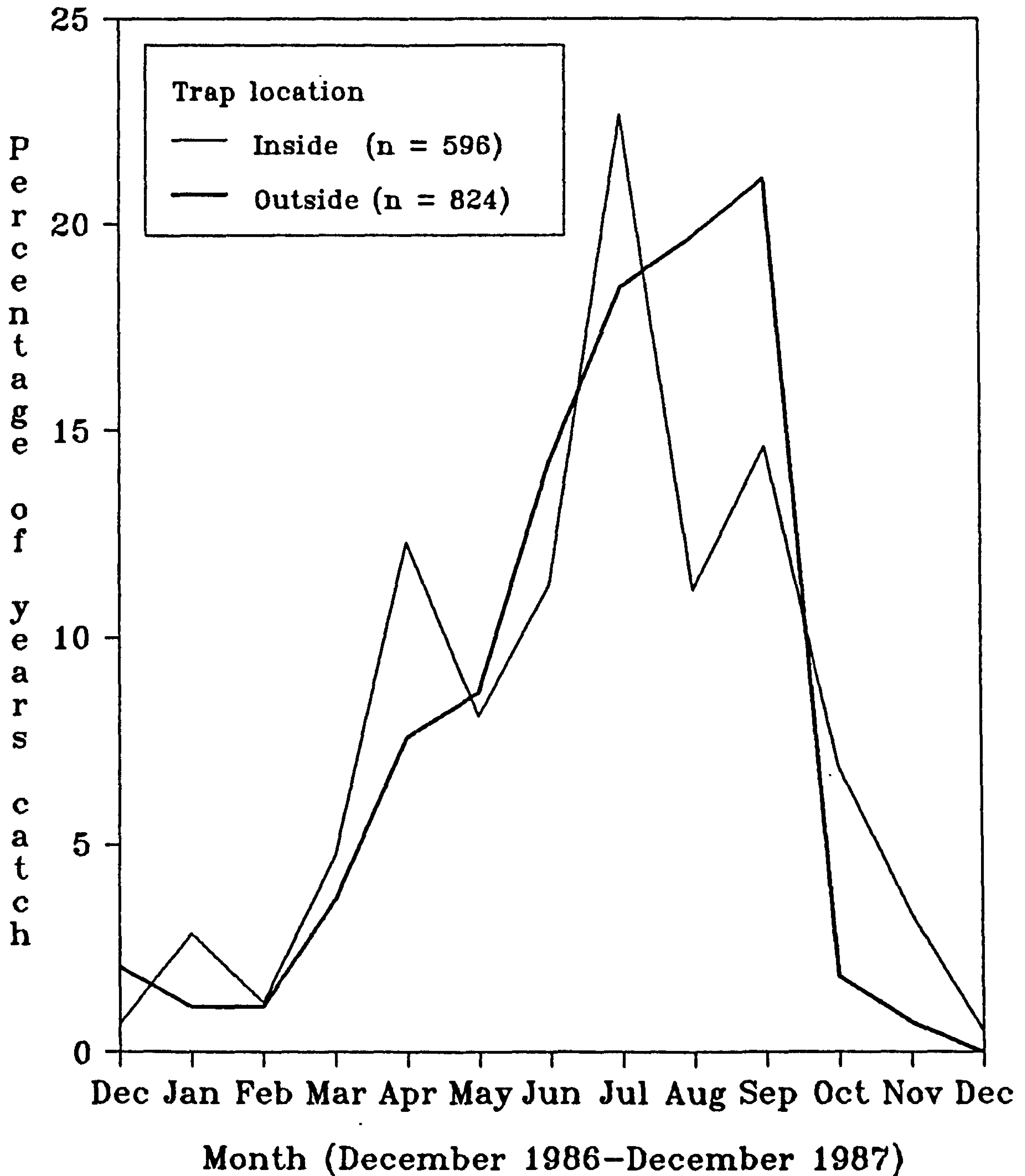
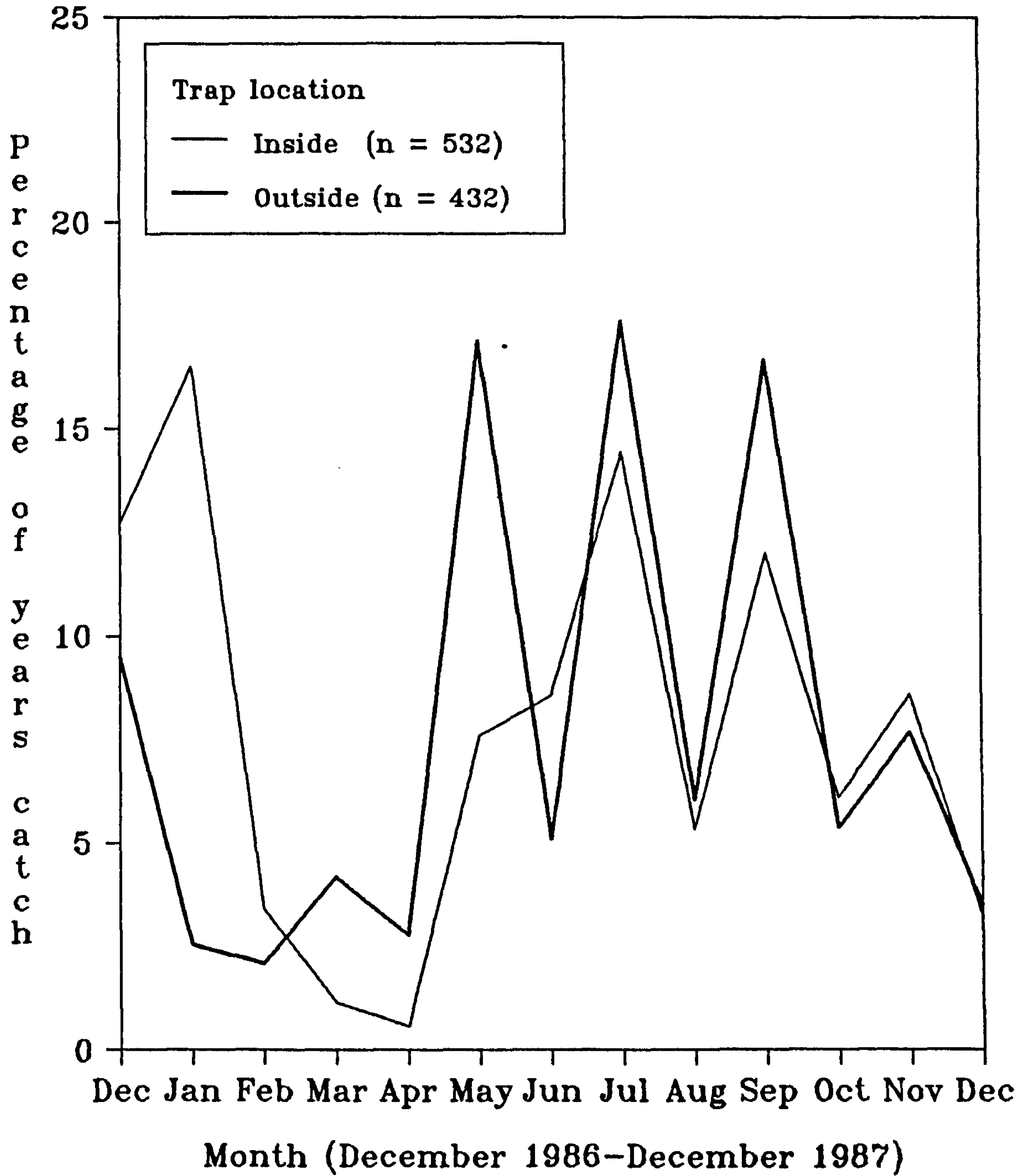


Fig. 46. Seasonal distribution of *Ph. bergeroti* at Bani–Thwa (Lowlands), (sticky traps, sexes combined)



two peaks in July and September while, at the same time, sticky traps (indoor and outdoor) (Fig.46) appear to show three peaks in May, July and September (zig-zag shape). The total catches in light traps were significantly higher than in sticky traps ($X^2 = 87.1$, 1df, $p < 0.001$).

An interesting observation was made in Ph.sergenti, the second abundant species in the lowlands, namely an early peak in May (Figure 47) and a late peak in September. The seasonality of Ph.alexandri presented in Figure 48 shows peaks in June to September but, as shown, the total catches are small in comparison to other major species.

The indoor and outdoor variations of the three species mentioned above are summarised in Figure 49.

The detailed data of sandflies collected are presented in Annexes 5 to 16.

5. Results of Dissection

As mentioned previously in the methodology, all the females caught by light traps were dissected and the total females dissected of each species in both regions are shown in Table 37. Two Ph.sergenti from the highlands were found to be infected with Leishmania tropica promastigotes (Plate 45). Details of the total dissected female Ph.sergenti are presented in Table 38. Plate 46 shows the ecology of sites where the infected Ph.sergenti have been found. The isoenzyme typing results are detailed in Chapter V, part 2, 2.3, [also see Al-Zahrani et al.(1988) in Annex 17].

In the lowlands two sandflies of the genus Sergentomyia,

S.squamimpleuris were found to be infected with epimastigotes (Plate 47). The identity of these epimastigotes could not be obtained and they did not grow in our *Leishmania* culture media.

Nematodes

Nematodes were seen in a number of sandflies; they were about 0.1 cm in length and pale coloured; a complete identification of these nematodes could not be made.

Mites

Phoretic mites were seen frequently on sandflies and they have been kept for a later study.

TABLE 37 Total number of female sandflies dissected in the period from December 1986 to October 1987.

Species	Number of females dissected Highland	Lowland	Total
Genus Phlebotomus			
sergenti	261*	56	317
arabicus	24	0	24
orientalis	7	0	7
alexandri	0	15	15
bergeroti	28	743	771
papatasi	0	5	5
Genus Sergentomyia	16	503+	519
Total:	336	1322	1658

*Two of these Ph. sergenti females were found to be infected with leishmania promastigotes (for further details see TABLE 38).

+Two of these Sergentomyia females (S. squamipleuris) were found to be infected with epimastigotes.

TABLE 38 Date and place of capture of Ph. sergenti infected with promastigotes in Asir Province (Highlands) of southwest Saudi Arabia.

Fly stock number	Date	Trap Type	Trap Site	Village	Collection Site Altitude	Collection Site Description of site
SSP286*	8/6/87	Light	Indoors	Shuhat	2000m	In deserted stone & mud house. The house was beside an animal house and 8m from an inhabited new concrete house. A farm surrounded all three buildings.
SSP454+	25/7/87	Light	Indoors	Mislat	2000m	Inside a deserted house near human dwellings, located at the centre of the village.

Notes:

173 Ph. sergenti sandflies were dissected during the season (June-August 1987).
261 Ph. sergenti sandflies were dissected during the year (December-October 1987).

*This was one of 4 female Ph. sergenti dissected on this day.
20 female Ph. sergenti were dissected in June 1987.

+This was one of 7 female Ph. sergenti dissected on this day.
99 female Ph. sergenti were dissected in July 1987.

Fig. 47. Seasonal distribution of *Ph. sergenti* (Lowlands), (light traps and sticky traps, sexes combined)

Fig. 48. Seasonal distribution of *Ph. alexandri* (Lowlands), (light traps and sticky traps, sexes combined)

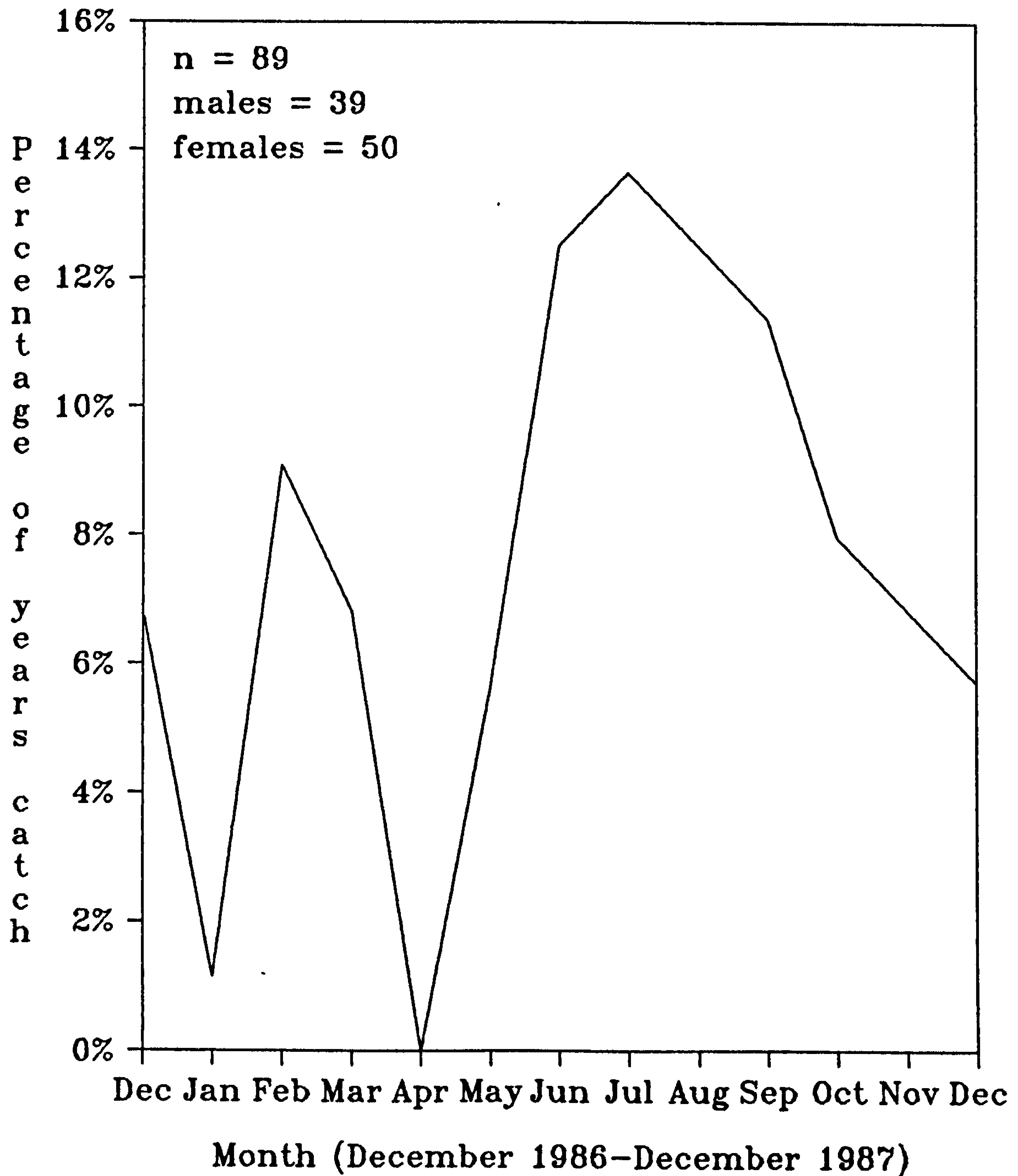
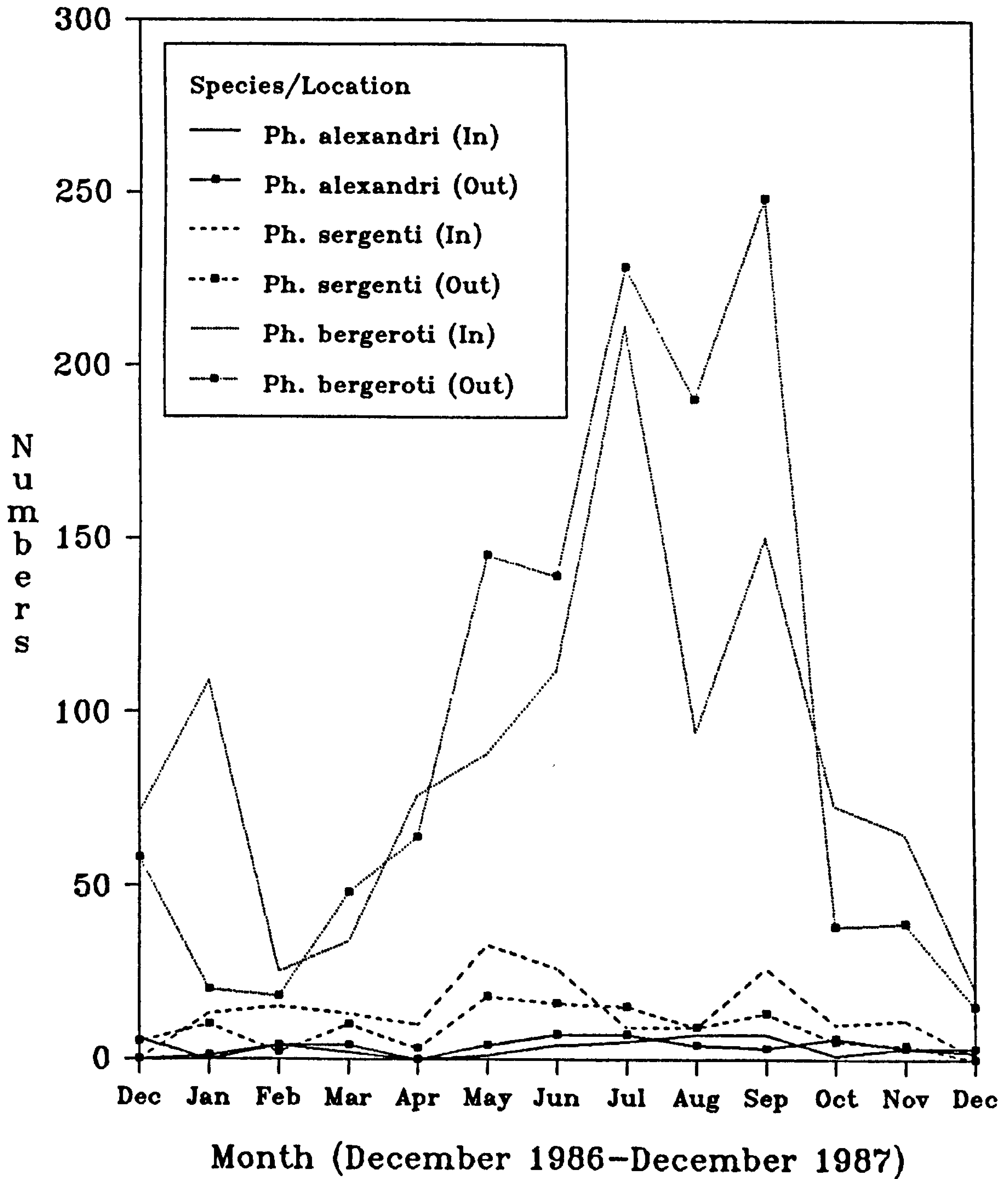
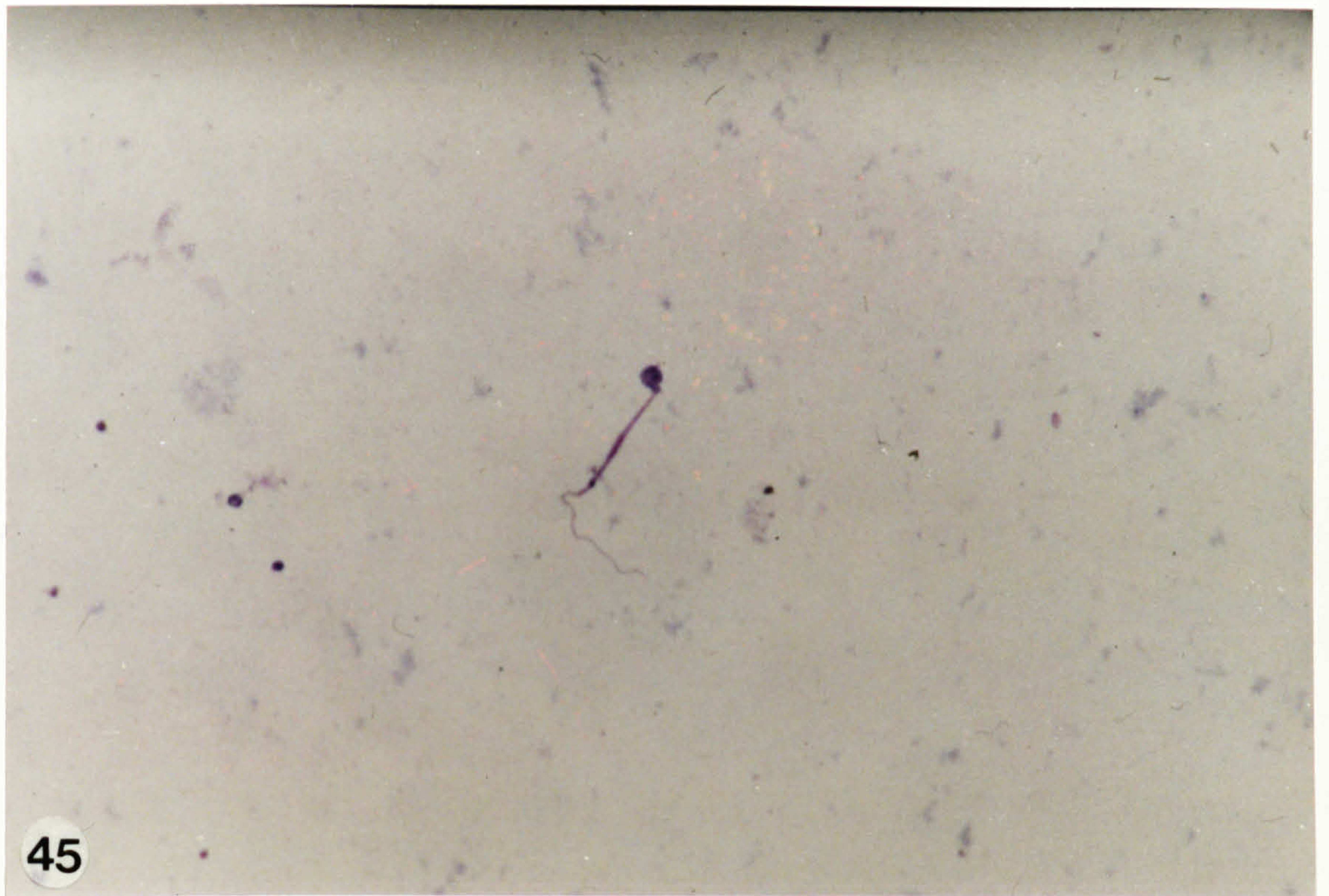


Fig. 49. Seasonal distribution of three species of the genus *Phlebotomus* (Lowlands), (light traps and sticky traps, sexes combined)



Infected *Phlebotomus sergenti* midgut

Plate 45. Promastigotes of *L.tropica* zymodeme LON-10 seen in midgut of *Ph.sergenti* in Shuhat area, Asir Province. (X 1600)


45

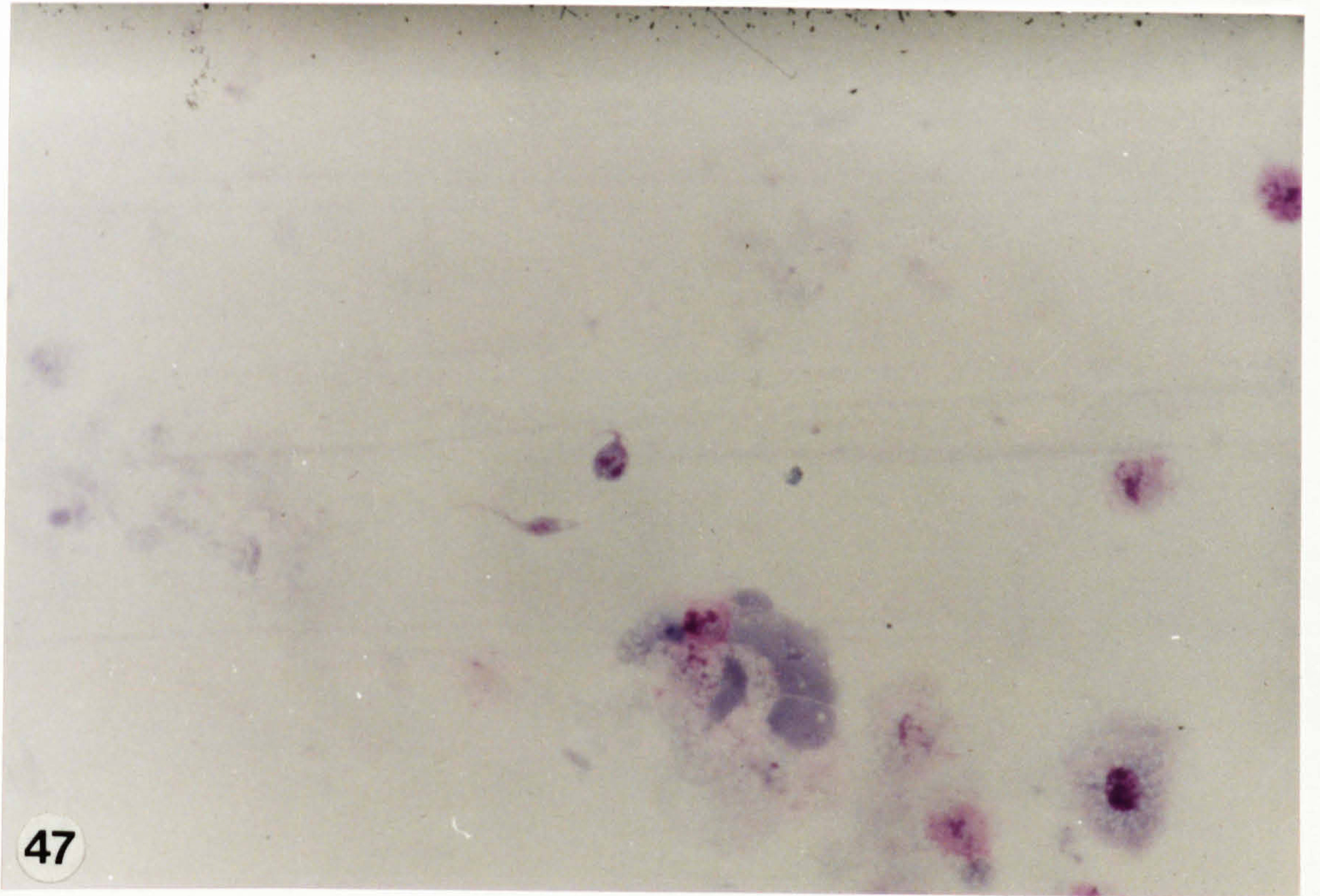
Ecology of *Phlebotomus sergenti*

Plate 46. House in Shuhat (Asir highlands) where an infected *Ph.sergenti* was captured. The parasite was typed as *L.tropica* zymodeme LON-10

46

Infected *Sergentomyia squamipleuris* midgut

Plate 47. Epimastigotes of kinetoplastid flagellate from midgut of _Sergentomyia squamipleuris_ (x 1600).


47

CHAPTER VI GENERAL DISCUSSION

1. Visceral Leishmaniasis

1.1 Parasite identity of human isolates

Before this study was carried out, the identity of parasites causing human VL was confirmed by isoenzyme typing of only two isolates, both from infants originating in Gizan Province (Peters *et al.* 1985). The parasite was designated as *L.donovani* *sensu lato*, zymodeme LON-42.

During the field work of this research, all 17 isolates from children suffering from kala-azar typed by the isoenzyme technique proved to be *L.donovani* *sensu lato* LON-42. As shown in Map no.11, these isolates originated from a wide geographical area and different altitudes. The isolate MHOM/SA/87/VL6, for example, was isolated from a child living in Khamismuter in the Mahayel district at an altitude 480 m above sea level, which is far removed from the Alkhoba district of Gizan province, about 400 km away at an altitude of 170m where the isolate MHOM/SA/87/VL57 was collected. The isolates MHOM/SA/87/VL51, MHOM/SA/87/VL54 and MHOM/SA/87/VL9 were from Yemeni patients who acquired the infection in their own country and came to Gizan province to seek treatment. Away from our study area Rioux *et* *al*.(1986) reported also the identification of *L.donovani* zymodeme MON-31 in two infants with kala-azar from lowland villages of the neighbouring Yemeni Arab Republic (MON31=LON-42). Taking

into account that a different parasite has been identified from dogs, this provides a unique epidemiological situation quite different from that in any visceral leishmaniasis foci. More isoenzyme typing of additional human and dog isolates will help towards a better understanding of the epidemiology. Ashford and Bettini (1987) stated "that in the Old World the taxonomy of L. donovani infantum based biochemical and/or clinical characteristics is still fluid. It is to be hoped that the typing of more isolates will sort out the confused epidemiological picture of the visceral leishmaniases".

The VL parasite was easy to grow and, in spite of practical difficulties in the field, 20 out of 60 (33.3%) cultures were positive. This is acceptable in such conditions and indicates how easy it is to grow this parasite. In bulk culture this parasite also grows quickly and easily in comparison to the parasite isolated from dogs (L.infantum, LON-49).

The infectivity of L.donovani LON-42 to hamsters can be considered as high since 3 out of 4 were found to be infected after they were injected intraperitoneally with positive cultures of this organism.

1.2 Possible vectors

During this study the search for a vector of VL was carried out in three different ways:

(i) regular collection and dissection of sandflies

from entomological fixed stations in the lowlands in kala-azar endemic villages.

(ii) night catches on human bait in the kala-azar endemic area with the collaboration of the malaria team working there.

(iii) regular collection from patients' houses in the Asir lowlands. The numbers and species of the genus Phlebotomus collected from each activity were summarised in Table 33. Moreover, in the lowland, a large number of female sandflies were dissected (total 1322), among which were 743 Ph.bergeroti (see Table 37).

In spite of this large number dissected, we failed to find any infected sandflies in the genus Phlebotomus. Because of this one can only postulate on the basis of the epidemiological background which species are the most probable vectors of VL in this area. Because Ph.orientalis which transmits visceral leishmaniasis in The Sudan occurs also in the southwest of Saudi Arabia, Lewis and Büttiker (1982) speculated that this sandfly might be the vector. However, this speculation is not valid for three reasons:- Firstly, the parasite of VL in the southwest of Saudi Arabia is L.donovani LON-42 which differs significantly from the visceralising parasite which has been isolated in The Sudan (Le Blancq and Peters, 1986) and one cannot assume that the vector is the same for the two organisms. Secondly, their assumption was based on their finding of Ph.orientalis in

the southwest but most of that collection was from the highlands of the area which is considered to be free of kala-azar transmission, and none of this species were collected from the Gizan area where active transmission of kala-azar takes place. Thirdly, in our collection from the kala-azar patients' houses we did not collect a single Ph.orientalis, nor were any collected from the human bait catchment area. In general only small numbers of this species were found. From the above points it is clear that this species is highly unlikely to be the vector.

Ph.sergenti were collected frequently in fixed stations, night catches and from patients' houses. In the course of this research, this species was incriminated by all the accepted criteria as the main vector of L.tropica, the etiological agent of cutaneous leishmaniasis in the highlands of the study area (see section 2 of this chapter and annex 17). From an epidemiological point of view, Pringle (1956) excluded this species as a vector of kala-azar in Iraq. It is most probable that Ph.sergenti is also implicated in L.tropica transmission in the lowlands. (Isolates from patients with CL in the lowlands have been collected and typed as L.tropica zymodeme LON-63). This belief is supported by the incrimination of the same species as a vector of L.tropica in the highlands of the study area. Assuming that this is true, in addition to the belief that "it is particularly interesting that a population of an Old World species of sandfly which readily supports development

of one parasite is unlikely to be a suitable host of another" (quoted from Killick-Kendrick,1979), one can only suggest that Ph.sergenti is not the vector of kala-azar in this area.

Ph.bergeroti is the dominant species in all our collections from the lowlands and has been collected in large numbers from all the patients' houses and human bait catches, implying that this species is both abundant and man-biting. Unfortunately this meets only part of the criteria for vector incrimination but, at the same time, one has no evidence to reject the possibility that this species may be as a vector of kala-azar in this area. In a personal communication Dr.R.Lane, said "I doubt very much that Ph. bergeroti is involved in VL transmission because of its close systematic relationship with Ph.papatasi" but mentioned it is very difficult to accept this absolutely since the role of Ph.papatasi in the transmission of VL in Iraq has still to be clarified (Lewis and Ward, 1987; Sukkar, 1985).

Ph.alexandri was collected in small numbers from human bait and patients' houses. The proportion in the lowland entomological fixed stations was only 3% of the annual total catch. This species has also been collected in the kala-azar endemic area of Oman where it is considered to be a potential vector of visceral leishmaniasis (R.Lane, personal communication). In our case, especially from human bait data, this speculation was difficult to reject and the role

of this species is left open for more entomological and ecological studies.

1.3 The role of the parasite found in dogs in the epidemiology of human visceral leishmaniasis in southwest Saudi Arabia.

Before this study was carried out and on the basis of the age distribution of VL cases, we made the working hypothesis that this area was like any other kala-azar endemic area in the Mediterranean region, that the dog was the secondary animal host for infantile visceral leishmaniasis and that the parasite found in infected dogs was the same as that in humans. As mentioned in the result section, the isoenzyme typing of isolates from three dogs proved that they were identical among themselves and indistinguishable from L.infantum zymodeme LON-49, the reference strain of Mediterranean human and dog infection. The isoenzyme typing of all 17 isolates from human patients corresponded to L.donovani sensu lato zymodeme LON-42 which differs significantly from L.infantum in five enzymes (MDH, ASAT, MPI, PEPD and NH).

The discovery of different organisms causing human and canine VL in the identical geographical areas in the study area was very surprising and raised several important questions. One of these questions is - can the dog parasites infect humans as well as dog, and did we simply fail to isolate any L.infantum from patients? To have a clear and complete answer is not easy. If we suppose that the dog is

the main animal reservoir for infantile VL and maintains a constant source of infection and that the vector feeds on both humans and dogs, it would transmit the same parasite with the same zymodeme, with perhaps a minimal intraspecific variation of the zymodemes. This is, however, not the case here and, in spite of characterising a fair sample of human isolates (17) by the isoenzyme technique, we could not find even one similar to that found in dogs. Furthermore, as we have shown, the infection rate among dogs is relatively high so that, if this parasite were to be transmitted to humans, it would be the common if not the only one (see annex 19).

The other explanation is that there may be a lack of man-dog-vector contact which is also possible, especially as we know that, in spite of using the dogs as guards in rural areas, the Muslim community does not accept having dogs in the houses. [Our prophet Mohammed, Peace be upon Him, said that Gabriel, Peace be upon Him, told him that the angels do not enter into a house where there may be dogs or their images. Today even a photograph of dogs is unacceptable]. In this case, it may mean that <u>L.infantum</u> is circulating between primary animal (natural) foci and that dogs form a secondary enzootic focus. This assumption is not easy to prove for two reasons. Firstly, the dogs in the endemic area are abundant and savage, continuously moving about near the human dwellings. On many occasions I saw dogs asleep just near the front doors of houses. Secondly, although we do not know the definitive vector, it is known that some sandflies

can travel for long distances (Killick-Kendrick _et al_.1984) which means that close contact between dog and man may not even be essential for transmission to occur.

Another possible explanation of this finding may be that the sandfly vector of _L.infantum_ bites man rarely or not at all. This can explain the lack of any VL zymodeme in common between humans and dogs. At this point one may ask what is the possible reservoir, either of primary (natural) foci and/or secondary zoonotic (synanthropic) foci. This question will be discussed in the following section.

1.4 Probable reservoirs of human visceral leishmaniasis.

There is general agreement between _Leishmania_ specialists about the zoonotic nature of infantile kala-azar which, as Ashford and Bettini (1987) pointed out, "has been held as a model for zoonoses, and has been much studied with this in mind". The stability of the disease, spatial and age distribution of the cases are good evidence that the pattern of disease is similar to that which occurs in the Mediterranean littoral which means, on the other hand, that this parasite should have a primary animal reservoir, man being a secondary host. If we assume that in southwest Saudi Arabia the parasite in dogs is _not_ transmitted to humans for the different reasons mentioned above, one must consider other peri-domestic and wild animals which are abundant in the area as possible zoonotic reservoirs.

The main peri-domestic animals in the kala-azar endemic

areas are sheep, goats, cows and cats. Without excluding any of these, none of which have been examined, it seems important to examine the possibility of cats as being domestic or primary reservoirs. Michael et al.(1986) who examined 80 cats collected in the Ismailiya Governate of Egypt found a strongly positive IHAT titre in three of them. Morsy et al.(1980) claimed that they found 20.5% of 78 stray cats in Amman, Jordan, infected with Leishmania amastigotes. Unfortunately these parasites were not biochemically typed, and both the reports of Michael et al.(1986) and Morsy et al. (1980) were from areas where kala-azar is not endemic or not reported. Thus the evidence for cats being domestic reservoirs of VL is far from significant and more studies are needed.

Wild animals such as the Arabian wolf (Canis lupus arabicus), red fox (Vulpes vulpes), jackal (Canis aureus), hedgehogs and porcupines are reported to exist in southwest Saudi Arabia (Harrison,1968) and have been seen frequently during the field work.

Porcupines were often seen near the farms, especially during the corn season in the autumn, and farmers in this area complain because they come to the farms and eat the corn. The farmers used to prevent this from happening by using nets or digging large pit traps around the farms in the direction from which the porcupines would appear. Bray (1974) considered this animal as one providing a true home for Leishmania infantum. This may be true if we take into

account the habits of this animal, and it may be that this animal could serve as a primary reservoir in the presence of suitable vectors which feed on both humans and porcupines, the transmission will be going on without the need for a secondary animal host.

Foxes also appear frequently near human dwellings and farms. The isolation of parasites from foxes in Italy and Portugal (Pozio *et al*.1981; Bettini *et al*. 1980; Abranches *et al*. 1984) encourages one to assume that these animals may play a role in the transmission of VL also in southwest Saudi Arabia.

The other wild animals are not commonly seen near human dwellings and their role (if any) in transmission is certainly less significant than the possible role of porcupines and foxes.

Many small mammals are abundant in the southwest of Saudi Arabia and were reported by Harrison (1968, 1972). These mammals are (i) rats (Muridae), *Arvicanthis niloticus*, *Rattus rattus*, *Rattus norvegicus*, *Mus musculus*, and *Acomys dimidatus*, (ii) gerbils (Gerbillinae), *G.nanus* and *G.cheesmani*. In the Sudan *Arvicanthis niloticus* and *R.rattus* were found to be infected with *Leishmania* (Hoogstraal and Heyneman,1969) and the *Arvicanthis* isolates were identical to some human isolates (Le Blancq and Peters, 1986a). However,in Ethiopia infected *Arvicanthis niloticus* have been reported but one of the isolates was different biochemically from the human VL parasites from the same locality (Chance

et al.,1978). A role of these and other animals in the transmission of VL in the southwest of Saudi Arabia might be assumed by analogy with these countries, but this is without any factual base. We have to avoid making such analogies since, for example, we have no epidemiological evidence that *Ph.orientalis* is a vector and we found different parasites in the dog and man. Without examining these rodents, their role in kala-azar transmission in this area must remain open. Discussing the nature of animal hosts of *L.infantum* and the existence of its primary or secondary hosts, Ashford and Bettini (1987) stated that - "Although wild and peri-domestic animals have been found infected and the black rat, *R.rattus* has been shown to be a potential reservoir host, it has not yet been demonstrated that a primary reservoir exists. Jackals and foxes have been found naturally infected but it has not yet been shown how non-colonial carnivores could be primary hosts of a parasite with a free-flying non host-specific vector. It is possible that fox or jackal earths provide an ideal habitat for sandflies, and that transmission occurs there, but this remains to be demonstrated. Indeed, it is possible that a large part of the distribution of *L.donovani infantum* is the consequence of human activity, and the ancestral origin of the parasites may have been in as yet undiscovered primary zoonotic foci far removed from the present day centres of distribution. Such primary foci may have been discovered in the Sudan, and could be present in Europe, in the form of inapparent

infections of wild vertebrates, but more work is required to confirm this.

1.5 Prevalence of canine visceral leishmaniasis

As detailed above (Chapter V, Part 3, section 2), 89 dogs were examined by various methods. Impression smears from the liver and spleen after careful examination revealed 6.1% positive. The result was confirmed when the tissue sections from the spleen and liver were stained by the immuno-peroxidase technique and these same dogs positive in the Giemsa stain were positive also with immunoperoxidase staining. This technique (immunoperoxidase) which has been reported to be more sensitive than Giemsa stain (Sells and Burton, 1981) has been used for tissue section staining to obtain more accurate results. However, as shown in Table 27, this technique in our studies did not yield a higher positivity rate than the Giemsa staining. The advantages of using immunoperoxidase staining was limited to confirming the results which were obtained by the Giemsa stain.

Blood spots from 88 dogs were examined by the Enzyme linked Immunosorbent Assay (ELISA) which was a more sensitive method, and 19.3% of these animals were found to be seropositive, indicating a high infection prevalence rate among the dogs examined. The intensity of infection can be assessed by the level of antibody titres which were relatively high as shown in Table 28. More than 58% (10 out of 17 seropositives) gave a positive titre of OD > 1.0 (OD value = optical density value in the ELISA

spectrophotometer reader).

1.6 Seroepidemiological survey in children

Among 706 blood spots examined by the ELISA, 26 samples (3.7%) were positive; among them only two samples were strongly positive with an OD value >1.0 while the majority (96%) gave considerably lower positive titres (0.4 - 0.8 OD value). Among these 42% were on the borderline between positive and negative values, which may indicate a low overall level of antibody titre. Whether these children were in the early stages of infection and could subsequently develop classical kala-azar symptoms, or whether they were in the chronic stage of subclinical infections is not easy to answer without a long-term follow-up. By prospective studies of subclinical forms of VL in children living in an endemic region of Brazil, Badaro et al.(1986b) found that, of a group of children classified as subclinical, 25% progressed to classical kala-azar within 5 months. The distribution of ELISA seropositive values by age showed no statistical correlation ($r = 0.0026$; $P = 0.99$). Nevertheless up to 50 samples from the age group 5 - 6 years were all negative, compared to 3.4% among the age group 4-5 years. This confirms the fact that this disease is restricted to children under 5 years of age. It also may reveal that the humoral response in kala-azar patients does not persist for a long time, probably less than one year (see Figures 22 & 23).

The distribution of ELISA-positive children by regions (locations) showed that the Noan region in Baha province had the highest rate, 9.3% of the total positives. This was surprising because VL cases in Baha province were much less common than in Gizan province where only 2.1% of the children were ELISA-positive. The ELISA-positives by provinces were 4.7%, 3.7% and 2.1% in Baha, Mahayel (Asir) and Gizan provinces respectively, while the numbers of reported cases were higher from Gizan, and Mahayel than from Baha province. It may be questioned whether these observations are due to differences in sample sizes since we have bigger samples from, for example, the Baha lowlands, or whether there is a difference in the susceptibility of children in different provinces, or if the diagnosis is better because of the greater awareness of medical staff in Gizan province. The latter seems to be a more logical and acceptable reason since all the people in the lowlands of the different provinces are similar in their life styles and socioeconomic conditions. However, this was a limited survey and indeed a larger follow-up and more organised prospective studies are needed in order to assess the true rates of patent and subclinical infections in this area.

1.7 Evaluation of Direct Agglutination Test (DAT) as a seroepidemiological tool in visceral leishmaniasis

When the blood spot samples were serologically examined, the ELISA technique was used as the main method of analysis because this technique has been widely shown for over a

decade by many workers to be a good and effective technique for seroepidemiological surveys. However, the recent report by Harith et al. (1986) on a modified Direct Agglutination Test (DAT) as a good economic and simple technique for seroepidemiological surveys of visceral leishmaniasis, suggested that it should have a part in the evaluation of blood spot samples.

As mentioned previously in Chapter IV, limited samples of blood spots collected from children and dogs during this survey were tested by the DAT as well as by the ELISA technique.

The results which were obtained by these two methods (ELISA and DAT) revealed that the DAT, at least on filter paper blood samples, was much less sensitive than the ELISA. Due to the availability of direct smear and culture data from dogs, it was considered better to take blood spots from dogs as examples. Of eight ELISA positive samples, four were also smear and culture positive. In the DAT test, one smear and culture positive sample was negative. In addition, two other ELISA positives, one of which was very strong (OD = 1.25), were DAT negative.

This negative result may be attributed to many factors. A main one which may hamper the specificity and sensitivity of this method as well as other serodiagnostic methods, is the antigen. Dr.D.Evans (personal communication) who is testing the impact of the antigen source on the sensitivity of this method, mentioned that different results are

obtained with different antigens and even the same antigen with different batches may give different results. Another factor which may contribute is the character of the filter paper used to collect blood spots. These samples were taken on Whatman filter papers and stored for more than a year at -20°C. According to the reports by Evengard *et al*. (1988) the resolution and the activity of immunoglobulin in filter paper blood samples were affected by time and temperature. Werner and Frosner (1985) reported that blood spots have only a limited value for seroepidemiological research and that low titre antibodies will not be detected. The last factor (the use of blood spots) may therefore be the main problem with the DAT. The DAT reported by Harith *et al*. (1986) was carried out on serum samples, Dr.El-Safi (a Ph.D student in the Department of Medical Parasitology), carried out this method (DAT) mainly with serum samples. The method was 100% sensitive and specific. However, as mentioned previously our study using filter paper samples was very limited and more studies on filter paper blood spots, as well as a large scale evaluation, are needed.

2. Cutaneous Leishmaniasis

2.1 Parasites and zymodemes

Of the forty four isolates from CL that were typed by the isoenzyme technique, 42 were characterised as *L.tropica* (5 zymodemes). The other two isolates, one from the Sudanese

patient and one from a Saudi patient living outside the Asir mountain range on the border with Riyadh province were L.major (2 zymodemes).

The five zymodemes seen among the 42 isolates showed a considerable intraspecific variation, as was reported for other isolates by Le Blancq and Peters (1986b).

The dominant zymodeme was zymodeme LON-72 which differs from the WHO marker (LON-7) in 4 enzymes (MPI, MDH, 6PGD and SOD). Zymodeme LON-73 consists of one isolate which differs from zymodeme LON-7 in 3 enzymes (MDH, 6PGD and SOD). LON-73 is similar to LON-72 but differs from it in MPI.

Zymodemes LON-71 and LON-10 included four CL isolates plus two isolates from sandflies (Ph.sergenti). They differ from each other in one enzyme (6PGD), and from the reference strain LON-7 in 8 of 12 enzymes (MPI, MDH, 6PGD, ASAT, ALAT, SOD, PK and NH).

Zymodeme LON-63 comprised three isolates which differ from LON-7 in 5 enzymes (MPI, MDH, 6PGD, PGM and SOD). The remarkable observation on this zymodeme is its limitation to widely separated foothill locations which, however, are within a narrow range of elevation (475-600m). These isolates are MHOM/SA/87/A129, MHOM/SA/87/A130 and MHOM/SA/87/122 which originated from Bel-Ghazi (Gizan province), Rejal-Alma and Mahayel (Asir province) respectively (see Map 10). The distinct character of these isolates, in spite of coming from widely separated sites may be due to their transmission by a different vector to that

found on the high plateau (about 2000m), or it might be a different subspecies of L.tropica. However, this is not easy to justify, and more isolates and genetic studies are needed. Neronov et al.(1986) in their English summary stated that "geographical insulation and environmental specialities were found to be important for the isolation of different Leishmania taxons. Taxonomy of cutaneous leishmaniasis needs further specification with regard to the structures of their distribution area."

Another point regarding enzyme variation concerns the two isolates from two sandflies near Abha City which gave two zymodemes (LON-10 and LON-71). As these two zymodemes were also identified in four human isolates, it is encouraging to postulate that this isoenzyme variation may contribute to the vector selective pressure (Le Blancq and Peters, 1986b).

2.2 Incrimination of Ph.sergenti as a vector of Leishmania tropica in the highlands of southwest Saudi Arabia

The speculation that Ph.sergenti is a vector of Leishmania tropica has been reported by different workers, among them Pringle (1957) from Iraq where L.tropica is endemic (Aljebori and Evans, 1980) and by Nadim et al. (1979b) from Afghanistan. Because the precise identity of the parasite found in this fly has apparently never been confirmed by biochemical or other critical method of typing, the question has remained open.

Our investigations have proved for the first time the identity of the promastigotes found in infected Ph.sergenti, and satisfy many of the criteria for the incrimination of vectors of leishmanias (Killick-Kendrick and Ward, 1981). The evidence for the incrimination of Ph.sergenti as a vector of Leishmania tropica in the southwest of Saudi Arabia is as follows:

(i) As mentioned in the results (Chapter V, Part 4), Ph.sergenti was the dominant species in the highlands, representing 59% of the total catch and 70% of total female catches. The presence of Ph.sergenti which is known to be an anthroponotic species as the dominant species in this area where cutaneous leishmaniasis is endemic, meets grades 1 and 2 of the vector incrimination criteria.

(ii) Of the total 261 female Ph.sergenti dissected throughout the season, promastigotes were found in large numbers in the midgut of 2 specimens.

(iii) The promastigotes from these two Ph.sergenti (ISER/SA/87/SSP286 and ISER/SA/87/SSP 454) were identified by the isoenzyme technique which confirmed that the organisms in each isolate were L.tropica, one corresponding to zymodeme LON-10 (SSP 286) and the other LON-71 (SSP 454). As mentioned above, LON-71 differs from LON-10 in one enzyme (6PGD) of 12 examined. In addition to their isolation from Ph.sergenti, L.tropica organisms belonging to zymodemes LON-71 (MHOM/SA/87/A126 and MHOM/SA/87/A127) and LON-10 (MHOM/SA/87/B635 and MHOM/SA/87/A137) were isolated from human patients from Asir and Baha provinces.

(iv) Experimental transmission of Leishmania by bites have been proved by Adler et al. (1938), cited by Ashford and Bettini (1987).

2.3 Disease and vector seasonal distribution

As shown previously in Chapter V, the Ph.sergenti population starts to increase in May, reaching the peak in July and August. The indoor catches started to decrease in August while the outdoor catch has another peak in October.

The infected sandflies found in June and July revealed that transmission can take place shortly after the population increases.

In comparison with Iraq, Abul-Hab and Al-Baghdadi (1972) reported that Ph.sergenti has two seasonal peaks, a small one in May-June with an indoor peak earlier than the outdoor peak. The second highest peak is in September.

The early peak for indoor catches in summer can be explained as follows. In summer the days are longer and the sandflies are attracted to traps inside during the early hours more than outside. The moon and other meteorological factors may also affect the sandfly activity. (Further discussion about seasonal variation and the factors that may affect this will follow below in section 3).

The seasonal distribution of cutaneous leishmaniasis cases seen in the Abha Leishmania clinic is shown with the corresponding Ph.sergenti population levels in Figure12 which shows that the cases diagnosed started to increase in September, reaching the highest numbers in December and

January and the minimum in August. The incubation period of L.tropica can be calculated as 8-12 weeks, based on the fact that we had infected flies in early June, and the first peak of cases diagnosed was in September. However, this prediction is not so accurate since it is known from Figure 18 that only 12% of patients came to the clinic within the first month after their lesion appeared, while the majority of patients came up to 5 months later.

2.4 Culture and animal susceptibility.

The experience gained from the cultures showed that L.tropica is an easy parasite to grow and there were no obstacles for its cultivation. Furthermore, some cultures, as mentioned in the results, persist more than four months and retain good motility. The sampling of specimens from patients using hypodermic needle aspiration which was found to be easy, practical and painless, gave better results than other techniques as long as there was no contamination. Cultures from sandflies (Ph.sergenti) were successfully carried out without the use of animal inoculation (especially as in our case there was no readily susceptible animal that could be used as a "biological filter"). The inevitable contamination in sandfly cultures was countered by the use of 50 ug/ml of gentamycin. The animal experiments showed that BALB/c mice were insusceptible and in hamsters the infections self-healed within four to six weeks.

2.5 Reservoir studies

Up to date the *L.tropica* reservoir is still debatable due to the negative finding for animal reservoirs. The suspected role of the dog as a reservoir in Afghanistan and Iran was not confirmed by further studies in our area. The working hypothesis is shared by *Leishmania* workers that man is the reservoir of *L.tropica*. Molyneux and Ashford, (1983) stated that "extensive unsuccessful attempts to discover animal reservoirs and the success of control methods assuming man-sandfly-man transmission have now convinced most people that man is the main host for *L.tropica*. It is thus an anthroponosis as opposed to a zoonosis". During the field activities of this study, only limited attempts could be made to collect animals from cutaneous leishmaniasis areas due to research objective priorities, and the large scale of the work. However, from field observations and during laboratory examination, it was noted that many cases of CL came from areas with no dogs and where rodent burrows were rare. Obviously this observation is not enough to contribute to any of the schools of thought about the animal reservoir of *L.tropica* and it leaves the question open for more field studies.

3. The Sandflies of Southwest Saudi Arabia - Abundance and Seasonal Variation

3.1 *Phlebotomus* species abundance and general ecology.

The relative abundance of each species in both regions

(high and lowlands) is shown in Figure 28. Some species were present such as Ph.sergenti, in both regions in fair numbers but other species were present in small numbers only and in limited regions, such as Ph.alexandri in the lowlands, and Ph.arabicus in the highlands. In relation to quantitative studies, many authors have pointed out that the method of sampling may affect the result and lead to wrong conclusions (Dergacheva et al., 1979; Molyneux and Ashford, 1983; Killick-Kendrick, 1987). Nevertheless, it is likely that all the local Phlebotomus species were represented in this survey. The reasons for this assumption are:

(a) In addition to the regular collections from fixed stations, random collections were carried out in different localities in the area, so that our sampling covered a wide geographical area.

(b) The sandfly collections were made by a variety of methods (light, sticky traps, aspiration in resting sites, night catches and room flitting by insecticide). This avoided the disadvantages of relying on any one method since no single method is of universal use. The abundance of individual species with their ecology is summarised as follows:

(i) Ph.sergenti

This species was collected in large numbers from the highlands of Asir Province, both from entomological fixed stations and mobile stations. It was collected from the inside and outside of deserted houses as well as from animal

shelters and inhabited human houses.

The villages where the collections were made were agricultural villages where most of the houses were located on the periphery of the farms. The main agricultural products were wheat in summer and corn and sorghum in the autumn.

Büttiker _et al_.(1982) and Lewis and Büttiker (1986) reported very small numbers (5 specimens) in two surveys from Asir province which led them to conclude that this species was rare. This is not true, firstly as shown by our collections in which a large number of this species were found and, secondly, because their survey was made during a short period in winter (March, 1979) when it was still cold and too early to see this species. Nadim _et al_. (1979) in his survey in Saudi Arabia covered neither the Asir mountains or lowlands. He made his collections in Bisha which is different ecologically from the Asir mountains. However, he collected this species from the Taif area which is a natural extension to the Asir mountains (Sarawut mountain).

The collection of _Ph.sergenti_ from the lowlands entomological fixed station at an altitude of 480 m above sea level is further evidence for its wide geographical distribution. Its biotope in the lowlands is somewhat different as described in Chapter II. However, the abundance of this species in the lowlands was low in comparison to that in the highlands. Further down in the lowlands at an

altitude of approximately 200 m above sea level, this species was absent from the collections.

Obviously the population sampling of this species and others was of the adult stage, and the search for breeding sites was not practical in this survey due to limited time during which other objectives of this project had to be carried out. Without doubt the breeding site is important in terms of epidemiology of disease and further investigations are needed on this species.

One more point related to Ph.sergenti is the possibility of the presence of Ph.saevus, which is closely related to Ph.sergenti as reported by Ashford (1974) in Ethiopia, and Lewis and Buttiker (1982) in Saudi Arabia. However, this latter species (Ph.saevus) has not been found among our specimens in spite of the fact that, during identification, the similarity between the two species was taken into account. Nevertheless, some samples of this species may be present and might have been missed during the identification process.

(ii) Ph.bergeroti

This species is dominant in the lowlands where it formed 35% of total catches from fixed stations. It was collected also from the highlands but in a low proportion (11.7%). The large number of this species collected from human bait and kala-azar patients' houses was remarkable and it might be proven later on in further studies that this species is implicated in the transmission of kala-azar or cutaneous

leishmaniasis in this part of the region.

Again at this point, it would be useful to compare the present findings with the sandfly survey which was carried out in Saudi Arabia by Büttiker and Lewis. Büttiker et al. (1982) caught 132 flies of this species from the lowlands (Ad Darb and Wadi Marraba), but Lewis and Büttiker (1986) mentioned that they collected only a single specimen from the lowlands. The same authors in 1982 stated that "P.bergeroti previously seemed a rare species but has recently numbered from 8 to 32% in three substantial collections". However, Nadim et al.(1979c) found this species was dominant in Mecca which is in the lowland part of the Western province, and he speculated that this species may be the vector for CL in that area.

The area from which this species was collected is rich in Acacia spp. and Tamarix spp.

(iii) Ph.alexandri

This occurs mainly in the lowlands, representing 3.2% of our total lowland Phlebotomus catches in the same area as Ph.bergeroti in collections from human bait and kala-azar patient surveys. It is ecologically probably similar to Ph.bergeroti. Lewis and Büttiker (1986) collected 317 specimens (with a high female proportion of 94.6%) in a total catch of 1231 (including Sergentomyia). These specimens were collected from the lowlands of the Western and Southwest regions. The authors described their sampling sites as "dry foothill biotope with comparatively dense

growth of trees and well established bushes of Acacias".

(iv) Ph.arabicus

This is a high altitude species and it was the second abundant one in the highland catches (20.6%). It was collected from the same places as Ph.sergenti. The male to female proportion was very high (4:1) Lewis and Büttiker (1982) reported that this species was present only in the highlands of the Southwest region.

(v) Ph.orientalis

Ph.orientalis was collected mainly from the highlands where it formed a low proportion (7.5%) of total highland catches. Few were collected from the lowlands. The role of this species as a possible vector for kala-azar in Saudi Arabia was discussed previously.

(vi) Ph.papatasi

In this survey we collected only a few specimens of this species (1.0%), all in the lowlands. In the mountain ranges of the highlands Ph.papatasi was absent. However, to the East of the Asir mountains at an altitude of 1150 m above sea level, this species was collected in large numbers during other activities from Bishah and Tathlaeth which have a different biotope with sandy soil and wide ranges of desert interspersed with old lava fields (harrats), with a high density of palm trees. Nadim et al. (1979c) reported that this species is dominant in the Bisha area where most probably it is the vector of CL due to L.major.

The rarity of this species in the mountain ranges in this survey can be confirmed by the findings of Lewis and Büttiker (1986) who reported only 1.2% of Ph.papatasi in their collections from the Asir plateau. Nadim et al.(1979c) also pointed out that this species had not been collected from Taif and Mecca which are topographically similar to the Asir high and lowlands respectively.

3.2 Seasonal variation

Although a little is known of some of the factors that influence the size of sandfly populations, this survey was organized and carried out in the field with efforts made to minimise any factors that may have biased the results, hoping thus to provide more realistic and informative data.

The seasonal distributions of each species are presented in detail in Chapter V. This section will be devoted to a discussion of the general conditions and criteria which have been used during this survey, in addition to the possible factors which may affect the present data. These are as follows:

(i) The data presented in this study were pooled from systematic sampling throughout the year, without any interruption.

(ii) This survey was carried on in two fixed stations in two different ecological areas, and in each fixed station three sampling sites were chosen. The traps used (light and sticky) were distributed equally in each site; furthermore in each site the traps were placed equally inside and

outside. This gave homogeneous data and a better chance of good sampling by minimising the effect of adult resting sites and their geographical distribution.

(iii) The samples from collection sites were made once a week which helped to minimise the affect of varying meteorological conditions such as rain, humidity, and wind speed.

(iv) The sandflies were collected by using fixed numbers of the two main traps commonly used of each species is presented graphically in different forms according to the type and site of traps, and according to the sex in some species. The available data provided a good source to test the effects of type and site of traps on the seasonal distribution.

We believe that the above measures have given added weight to the data collected in this survey. However, for a critical examination of the data, the following observations should be pointed out:

(a) in the highlands where Ph.sergenti is dominant, the total catches in light traps were significantly higher than in sticky traps (X^2 = 98 Idf p <0.01) while in the lowlands, where it is not dominant, the total populations in the two traps were equal. The same applies to Ph.bergeroti in the lowlands where it was the dominant species, the total catches in the light traps being significantly different from the sticky traps (X^2 = 87.2 Idf p<0.01) while, in the highlands where it is not the dominant species, they show no

significant differences between the light and sticky traps (X^2 = 0.1 Idf p>0.5).

The explanation of this observation may be contributed to the competition of sandfly population for attraction to the light and that the high proportion of species will have more chance to be presented in the trap.

(b) The total catches of <u>Ph.sergenti</u> in indoor collections were significantly higher than the total catches from outdoors (X^2 = 37.6 Idf p <0.01) (X^2 = 16.8 Idf p <0.01) in both highlands and lowlands respectively. In contrast, the total of <u>Ph.bergeroti</u> catches from outside were significantly higher than the indoor catches in both regions, lowland and highland (X^2 = 6.8 Idf p<0.01; X^2 = 5.4 Idf p<0.02). These important differences encourage us to conclude that <u>Ph.sergenti</u> prefers to rest indoors and furthermore that this species may have a tendency to be an endophagic biter, contrary to <u>Ph.bergeroti</u> which has a tendency to be exophilic. We need to stress, however, that this might still not be a significant difference since other factors such as meteorological conditions, the appearance of the moon and human errors during sampling activities may be contributing to these results.

The other species were present in small numbers only and their seasonal variations are interpretable.

3.3 <u>Genus Sergentomyia spp.</u>

These species were collected in very high numbers and

they represented 41.8% of total catches in fixed stations (2840 specimens out of 6797). The female proportion was 55% of the total Sergentomyia specimens.

The specimens collected were mainly from the lowlands which have a similar biotope to the African side of the Red Sea (Larsen, 1984).

The two females of S.squamipleuris infected with epimastigote forms revealed that enzoonotic infection with kinetoplastids in this area is endemic. Unfortunately, enzoonotic disease surveillance in Saudi Arabia in general, and in the southwest in particular, have been ignored so far. No survey has been carried out but it is hoped that this present study will lead the way to more detailed field studies in this area.

4. Leishmaniasis Control and Research Required in Saudi Arabia

4.1 Present situation of leishmaniasis control

Without doubt the control and/or eradication of any disease should be the target of any disease study. In order to apply proper control measures, the information available must include a knowledge of disease epidemiology. Vector biology and reservoir hosts must be considered in any control plan.

The complexity of the epidemiology of the leishmaniases due to the wide diversity of parasites and lack of information on their vectors and resevoirs, have hampered

most of the trials which have been carried out for their control. However, the degree of success in control measures varies from country to country and from species to species of the pathogens. The best example of this is L.tropica, the causative agent of anthroponotic cutaneous leishmaniases (ACL) which has been eradicated from the USSR by the treatment of patients and spraying against sandflies with DDT, whereas L.major, the causative agent of zoonotic cutaneous leishmaniases (ZCL), has so far not been controlled in the USSR in spite of wide and varied studies which were carried out in endemic foci (Kellina, 1981, Saf'Janova 1971).

Generally speaking, four types of control measures are widely used and have been tried in different countries. These methods are:

(i) active case reporting and treatment

(ii) reduction of sandfly vector population by spraying with suitable insecticides

(iii) destruction of animal reservoirs by available and suitable methods, such as the destruction of rodent burrows or shooting of stray dogs.

(iv) by vaccination with live promastigotes. This has been carried out in Israel and the USSR on a limited scale with many disadvantages. New vaccination trials with an avirulent parasite (L.arabica) against L.major are currently being undertaken (W.Peters, personal communication).

In practical terms, these methods have been valuable and effective in areas where the epidemiological structure of

endemic foci and the transmission mechanisms are known.

Due to public demand, arbitrary control measures have had to be carried out in most of the provinces of Saudi Arabia, using mainly two methods, insecticide house spraying and case treatment through PHC's. Unfortunately these methods were applied without any epidemiological or biological basis. Recent information from the Al-Ahsa oasis in the Eastern provinces and from the southwest of the country may provide a guide for better, rational control measures.

4.2 Obstacles facing Leishmania control in Saudi Arabia

The obstacles facing the control of leishmaniases in Saudi Arabia can be summarised as follows:

4.2.1 Shortage of expertise and trained personnel

A shortage of expertise in leishmaniasis is worldwide. In Saudi Arabia the shortage of expert personnel is the main problem facing leishmaniasis control. In the control programme which was recently started, most national workers joining the scheme lacked any proper experience.

4.2.2 Lack of basic epidemiological information about the disease and its animal and vector biology

A part of the Al-Ahsa oasis has yielded valuable information about ZCL, its vectors and reservoirs (Elbihary *et al*.,1987; Peters *et al*.,1985; Killick-Kendrick *et al*., 1985) and recently the Southwest during the current investigation (Peters and Al-Zahrani, 1987; Al-Zahrani *et*

al.,1988; Al-Zahrani, Peters and Evans, in press). The other endemic provinces have not been covered by any studies.

4.2.3 Cases improperly diagnosed

Most cases, particularly those in the Primary Health Care Centres, are diagnosed on the basis of clinical manifestations, with laboratory diagnosis made sometimes on direct smears but without any culture being made in most hospitals. Therefore no isolates were collected from patients for biochemical typing. A correct diagnosis and parasite identification should be taken into account in any attempt at data interpretation.

Kala-azar in endemic areas is diagnosed at best on a clinical basis and by bone marrow smears in some hospitals only. Sensitive methods, (eg serodiagnosis by ELISA, IFAT and DAT) are not used for diagnosis. As Kala-azar is endemic in the areas where malaria and schistosomiasis are also endemic, misdiagnosis may occur and unfortunately, early diagnosis will be missed by many physician in the PHC's.

4.2.4 Reporting system

In spite of the obligatory reporting system for leishmaniasis, the recording of data is still deficient in some areas of The Kingdom. The main reasons for this deficiency are:

(a) heavy overload of work of some physicians

(b) misdiagnosis as a result of the inexperience of physicians

(c) reluctance of people to seek treatment until the disease has become particularly troublesome.

4.2.5 Absence of case mapping and proper data analysis

Monthly reports of leishmaniasis cases and entomological activities are submitted to the *Leishmania* Department of the Ministry of Health. Data on name, age, sex, nationality, address, and history are collected, but, without any proper analysis no infection focus mapping has been done. Therefore the endemicity of the disease and the endemic foci are not precisely known.

4.3 Studies required to elucidate the endemic leishmaniases foci in the country

4.3.1 Visceral leishmaniasis

(i) Parasite identification -

Although our study provides a light for the better understanding of the type of VL parasite, further biochemical typing of VL isolates from all the endemic foci, especially from the other parts of the lowlands which were not covered by this study, or any other endemic area needs to be carried out. More extensive biochemical typing of isolates will give a better understanding of the nature of the parasites causing VL in Saudi Arabia.

(ii) Animal reservoirs and vectors -

The reservoir host(s) and sandfly vector(s) of VL are still unknown. During this study attempts made to find them

failed and our speculation on suspected animals and sandfly species were based only on some epidemiological observations. Therefore detailed studies on the reservoirs and vectors are still urgently needed.

(iii) Characterization of endemic foci structure - This requires proper case mapping, records of the social activity of people in this area and all other aspects related to disease transmission including the necessary information mentioned above.

(iv) Seroepidemiological surveys among the populations at risk will help to assess the degree of endemicity of VL and the ratio of subclinical to clinical infections.

(v) Clinical studies on the disease have not been carried out except for a few limited studies which were reported among patients seen in hospitals outside endemic areas (El-Behairy *et al*., 1982).

4.3.2 Cutaneous leishmaniases

(i) Biochemical characterization of etiological agents in endemic foci.

Apart from the Al-Ahsa oasis and the highlands of the Asir and Baha provinces, the distribution of endemic foci of*L.tropica* and *L.major* is incompletely known due to the absence of biochemical characterization of isolates. More characterization of isolates from patients will help to map the geographical distribution of both parasites. This step should not be ignored since the control measures against

each of them are different and one species (L.major) is far more difficult to control than another (L.tropica).

(ii) Reservoir and vector studies

The previous studies in the Eastern province (Al-Ahsa oasis) have incriminated the vectors and the reservoirs of L.major in that area. The present field research carried out in the Southwest has provided answers to some epidemiological aspects of CL in this area. Surveillance in other endemic areas for vectors and reservoirs has still to be made since different areas may have different vectors and reservoirs.

4.4 Recommendations for effective and economic control measures in Saudi Arabia

4.4.1 Urgent control measures

(i) The Ministry of Health has the opportunity of applying trial of control based on a sound epidemiological understanding of the Al-Ahsa oasis focus of ZCL using a rational approach, ie, environmental change by destroying rodent burrows, and all the criteria for the evaluation of this method are available. This procedure has been recommended by the National Leishmania Research Programme based at King Faisal University in the Eastern province which is supported financially by KACST (King Abdulaziz City for Science and Technology).

The programme team there carried out a series of studies which yielded very valuable information, but, for unknown reasons, the pilot control project was suspended. I have referred to this point to emphasise that this would be the proper place to test properly designed, scientifically based measures to gain better practical experience of CL control.

(ii) Case diagnosis involving microscopic examination, as well as culture, should be made available in each hospital and major PHC. This will give a better understanding of the prevalence of disease and also a chance to collect isolates from different regions for biochemical typing (eg, in one of the WHO Reference Centres).

(iii) Precise case mapping, especially for visceral leishmaniasis, will allow a better understanding of its geographical distribution.

(iv) The spraying of insecticides should be limited to known L.tropica - endemic areas based on case investigations. Random spraying is ineffective and uneconomical.

(v) Sandfly collections from fixed stations and the dissection of flies are easy tasks which should be carried out especially in areas

where the vector is unknown.

(vi) The Malaria Training Centre in Gizan province was originally opened to instruct local malaria workers, but has subsequently been considered by WHO as a Regional Training Centre. This Centre may help with <u>Leishmania</u> control as well as malaria since both diseases are endemic in this area, by conducting short courses (4-6 weeks) under the supervision of leishmaniasis experts with the cooperation of WHO.

4.4.2 <u>Long-term control strategy</u>

(i) The key for the solution to most of the previously mentioned obstacles is to have a well qualified, trained team, either in the headquarters of the Leishmaniasis Control Department or in the peripheral centres of the provinces. This can be achieved by extensive training, either locally or abroad, through collaboration between the Ministry of Health and academic centres and organizations, eg, University Institutes and WHO.

(ii) The current activities of the Leishmaniasis Control Team consist mainly of data collections from hospitals and PHC's with inadequate entomological activity and with random, arbitrary

spraying in some areas. Obviously no real laboratory or field investigations have been carried out and, subsequently, no good facilities for such investigation have been available so far. The optimal control planning and evaluation will not become possible without having a central reference laboratory covering major areas such as disease diagnostic methods, entomological research, laboratory and animal experiments.

(iii) Completion of the research and studies related to leishmaniasis mentioned previously are the best approaches for any effective control measures, and long policy control strategies should be based on effective and permanent measures. Obviously the required studies are more than the capacity of the present *Leishmania* department could cope with. *Leishmania* control and eradication are national targets and other departments and government agencies should be encouraged to take part in such a programme. The following points are examples of how such large-scale research could be carried out. These are:

(a) A Department of Research should be established in the Ministry of Health with a special budget to support and finance local scientists to

carry on research on diseases related to the country. Research on the leishmaniases should be among these priorities.

(b) The best example of national integration in leishmaniases control is the NLRP/KFU programme which received generous financial support from KACST, but more is needed to continue that research. The officially authorized personnel in the Ministry of Health should collaborate and confer with KACST for further grants for leishmaniasis studies.

(c) The National Assembly for Environmental Protection and Animal Conservation covers animal collections across the country by well trained biologists. We need to collaborate with them to help in the collection of animals, training and carrying on common research which would be useful for both sides.

(d) The role of the Universities for staff promotion requires that certain numbers of research projects should be carried out and this would be an opportunity for an exchange of information between the Ministry of Health and any University staff members willing to carry out research on leishmaniasis.

With the end of these recommendations I would like to

emphasize again the point that the necessity of meeting public demand for control should not make us forget that continuing field studies would be more valuable in the long term for the public and future generations.

4.5 Conclusions

This study provides new, exploitable knowledge about the leishmaniases in previously unresearched areas in Saudi Arabia. It contributes and elucidates additional information to knowledge on the leishmaniases in general.

The etiological agents of both visceral and cutaneous leishmaniasis in the southwest of Saudi Arabia have been identified and biochemically characterized. The vector (*Ph.sergenti*) of cutaneous leishmaniasis in the highlands has been incriminated by nearly all the required criteria. New facts on the relationship between the human VL and canine VL have emerged during the field studies of this research, in addition to other field and laboratory experiences which I hope will contribute to the main target which is disease control.

REFERENCES

Abdel-Fattah, A., Ismail, M.A. & Morsy, T.A., 1982. Clinical studies on cutaneous leishmaniasis in Saudi Arabia. Journal of the Egyptian Public Health Association 59: 51-66.

Abdel-Wahab, R.M., Morsy, T.A. & Essa, M.H., 1985. Clinical and laboratory aspects of visceral leishmaniasis in Gizan, Saudi Arabia. Journal of the Egyptian Society of Parasitology 14: 563-572.

Abranches, P., Conceicao-Silva, F.M. & Silva-Pereira, M.C.D., 1984. Kala-azar in Portugal. V. The sylvatic cycle in the enzootic endemic focus of Arrabida. Journal of Tropical Medicine and Hygiene 87:197-200.

Abul-hab, J. and Al-Bagdadi, R., 1972. Seasonal occurrence of man-biting Phlebotomus (Diptera : Psychodidae) in the Baghdad area, Iraq. Annals of Tropical Medicine and Parasitology 16:165-166.

Adler, S., 1936. Canine visceral leishmaniasis with special reference to its relationship to human visceral leishmaniasis. Report Communicated to the 3rd International Congress of Comparative Pathology, Athens. (15-18 April, 1936), 4-14.

Adler, S., 1964. Leishmania. In: Advances in Parasitology, Volume 2, (Edited by Dawes, B.), Academic Press, London and New York. pp. 35-96.

Adler, S. & Theodor, O., 1929. The distribution of sandflies and Leishmania in Palestine, Syria and

Mesopotamia. Annals of Tropical Medicine and Parasitology 23:269-306.

Adler, S. & Theodor, O., 1930. The inoculation of canine cutaneous leishmaniasis into man and the behaviours of various strains of Leishmania in mice. Annals of Tropical Medicine and Parasitology 24:197-210.

Adler, S. & Theodor, O., 1931. Investigation on mediterranean kala-azar. I. Introduction and epidemiology Proceedings of the Royal Society, London, Series B 108:447-453.

Al-Blehed, A.S., 1982. Aspects of emergence and change in Asir. PhD Thesis, University of Southampton, pp. 126-144.

Al Dafas, A.A. & Mohammed, C.K., 1985. The epidemiology of cutaneous leishmaniasis in ARAMCO health care population. In: Medical Symposium on Leishmaniasis. March 22-24, 1980, Dammam. Ministry of Health, Riyadh, pp. 147-160.

Al-Gindan, Y., Abdul-Aziz, O. & Kubba, R., 1985. Some clinical aspects of cutaneous leishmaniasis in Al-Hasa, Saudi Arabia. In: Medical Symposium on Leishmaniasis, March 22-24, 1980, Damman. Ministry of Health, Riyadh, pp. 57-72.

Al-Gindan, Y., Omer, A.H.S., Al-Humaidan, Y., Peters, W. & Evans, D.A., 1983. A case of mucocutaneous leishmaniasis in Saudi Arabia caused by Leishmania major and its response to treatment. Clinical and Experimental Dermatology 8:185-188.

Aljeboori, I., Tarik, I. & Evans, D.A., 1980. Leishmania spp. in Iraq. Electrophoretic isozyme patterns. II. Cutaneous leishmaniasis. Transactions of the Royal Society of Tropical Medicine and Hygiene 74:178-184.

Al-Taqi, M.S., 1978. Studies on leishmaniasis in Kuwait. PhD Thesis, University of London, pp. 392.

Al-Taqi, M.S. & Evans, D.A., 1978. Characterisation of Leishmania spp. from Kuwait by isoenzyme electrophoresis. Transactions of the Royal Society of Tropical Medicine and Hygiene 72:56-65.

Al-Zahrani, M.A., Peters, W. and Evans, D.A., 1988. Visceral leishmaniasis in man and dogs in southwest Saudi Arabia. Transactions of the Royal Society of Tropical Medicine and Hygiene (in press).

Al-Zahrani, M.A., Peters, W., Evans, D.A., Ching, C., Smith, V. & Lane, R.P., 1988. Phlebotomus sergenti, a vector of Leishmania tropica in Saudi Arabia. Transactions of the Royal Society of Tropical Medicine and Hygiene 82:416.

Antunes, F., Carvalho, C., Tavares, L., Botas, J., Forte, M., Delrio, M.A., Dutschmann, L., Costa, A., Abranches, P., Pereira, S.C., Paiva, J.E.D., Araujo, F.C. & Paptista, A., 1987. Visceral leishmaniasis recrudescence in a patient with AIDS. Transactions of the Royal Society of Tropical Medicine and Hygiene 81:595.

Arias, J.R., Miles, M.A., Naiff, R.D., Povoa, M.M.,

DeFreitas, R.A., Biancardi, C.B. & Castellon, E.G., 1985. Flagellate infection of Brazilian sandflies (Diptera : Psychodidae): isolation of endotrypanum and Leishmania. American Journal of Tropical Medicine and Hygiene 34:1098-1108.

Ashford, R.W., 1974. Sandflies (Diptera:Phlebotomidae) from Ethiopia: taxonomic and biological notes. Journal of Medical Entomology 11:605-616.

Ashford, R.W. & Bettini, S., 1987. Ecology and epidemiology: Old World. In: The Leishmaniases in Biology and Medicine, Volume 1 (Edited by Peters, W. & Killick-Kendrick, R.), Academic Press, London, pp. 365-424.

Bada, J.L., Arderiu, A., Gimenez, J. & Gomez-Asha, J.A., 1979. Pancytopenia in kala-azar. Transactions of the Royal Society of Tropical Medicine and Hygiene, 73:246.

Badaro, R., Reed, S.G., Barral, A., Orge, G. & Jones, C.T., 1986a. Evaluation of the Micro Enzyme-Linked Immunosorbent Assay (ELISA) for antibodies in American visceral leishmaniasis; antigen selection for detection of infection-specific responses. American Journal of Tropical Medicine and Hygiene, 35:72-78.

Badaro, R., Jones, T.C., Lorenco, R., Cerf, B.J., Sampaio, D., Carvalho, E.M., Rocha, H., Teixeira, R. & Johnson, W.D., 1986b. A prospective study of visceral leishmaniasis in an endemic area of Brazil. Journal of Infectious Diseases, 154:639-649.

Badaro, R., Jones, T.C., Carvalho, E.M., Cerf, B.J., Sampaio, D., Reed, S.G., Barral, A., Teixeira, R. & Johnson, W.D., 1986c. New perspectives on subclinical form of visceral leishmaniasis. Journal of Infectious Diseases, 156:1003-1011.

Barker, D.C., 1987. DNA diagnosis of human leishmaniasis. Parasitology Today, 3:177-184.

Barker, D.C., Arnot, D.E. & Butcher, J., 1982. DNA characterization as a taxonomic tool for identification of kinetoplastic flagellate protozoans. Biochemical characterization of Leishmania, (Edited by Chance, M.L. and Walton, B.C.), UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, Geneva, pp. 139-180.

Berens, R.L. & Marr, J.J., 1978. An easily prepared defined medium for cultivation of Leishmania donovani promastigotes. Journal of Parasitology, 64:160.

Bettini, S., Gradoni, L. & Pozio, E., (1978). Isolation of Leishmania strains from Rattus rattus in Italy. Transactions of the Royal Society of Tropical Medicine and Hygiene, 72:441-442.

Bettini, S., Maroli, M. & Gradoni, L., 1981. Leishmaniasis in Tuscany (Italy): (IV). An analysis of all human recorded cases. Transactions of the Royal Society of Tropical Medicine and Hygiene, 75:338-344.

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Bettini, S., Pozio, E. & Gradoni, L., 1980. Leishmaniasis in Tuscany (Italy): (II). Leishmania from wild Rodentia and Carnivora in a human and canine leishmaniasis focus. Transactions of the Royal Society of Tropical Medicine and Hygiene, 74:77-82.

Bienzle, U., Ebert, F. & Dietrich, M., 1978. Cutaneous leishmaniasis in Eastern Saudi Arabia. Epidemiological and clinical features in a non-immune population living in an endemic area. Tropenmedizin und Parasitologie, 29:188-193.

Bradley, D.J., 1987. Genetics of susceptibilty and resistance in the vertebrate host. In: The Leishmaniases in Biology and Medicine, Vol. 2. (Edited by Peters, W. & Killick-Kendrick, R.), Academic Press, London, pp. 551-581.

Bradley, D.C. & Kirkley, J., 1977. Variation in susceptibility of mouse strains to Leishmania donovani infection. Transactions of the Royal Society of Tropical Medicine and Hygiene, 56:527-528.

Bray, R.S., 1974. Leishmania. Review of Microbiology, 28:189-210.

Bray, R.S. & Dabbagh, M.A., 1968. Investigations into the epidemiology of leishmaniasis. Unsuccessful search for the reservoir host of kala-azar in Baghdad. Journal of Tropical Medicine and Hygiene, 71:46-47.

Brewer, G.J., 1970. An Introduction to Isoenzyme Techniques. Academic Press, New York, San Francisco, London.

Büttiker, W. & Lewis, D.J., 1979. Ecological studies at Hofuf, Eastern Saudi Arabia, in relation to dermal leishmaniasis. Tropenmedizin und Parasitologie, 30:220-229.

Büttiker, W., Al-Ayed, I.H., Alwabil, A.H., Assalby, H.S., Rashed, A.M. & Shareefi, O.M., 1982. Medical and applied zoology in Saudi Arabia. A preliminary study on leishmaniasis in two areas of Asir Region. Fauna of Saudi Arabia, 4:509-519.

Büttiker, W., Indonisi, H., Seith, M.E. & Turkestani, A., 1980. Medical and Applied zoology in Saudi Arabia. A study on cutaneous leishmaniasis in Riyadh District. Fauna of Saudi Arabia, 2:419-426.

Chance, M.L., 1979. The identification of Leishmania. In: Problems in the Identification of Parasites and their Vectors, (Edited by Taylor, A.E.R. & Muller, R.), Blackwell, Oxford, pp. 55-74.

Chance, M.L., Schnur, L.F., Thomas, S.C. & Peters, W., 1978. The biochemical and serological taxonomy of Leishmania from the Aethiopian zoogeographical region of Africa. Annals of Tropical Medicine and Parasitology, 72:533-542.

Chang, K.P. & Fish, W.R., 1983. Leishmania. In: In vitro cultivation of protozoan parasites, (Edited by Jensen, J.B.), Boco Raton, Florida, USA, C.R.C. Press, pp. 112-153.

Chowdhary, S., 1976. Bone-marrow smear of a boy aged 4 years from Riyadh, showing L.donovani (laboratory demonstration). Transactions of the Royal Society of

Tropical Medicine and Hygiene, 70:23.

Clauvel, J.P., Couderc, L.J., Belmin, J., Daniel, M.T., Rabian, C. & Seligmann, H., 1986. Visceral leishmaniasis complicating acquired immunodeficiency syndrome (AIDS). Transactions of the Royal Society of Tropical Medicine and Hygiene, 80:1010-1011.

Cuba Cuba, C.A., Marsden, P.D., Barreto, A.C., Rocha, R., Sampaio, R.R., Patzlaff, L., 1981. Parasitologic and immunologic diagnoses of American (mucocutaneous) leishmaniasis. Bulletin of the Pan American Health Organization, 15:249-259.

Cuba Cuba, C.A., Miles, M.A., Vexenat, A., Barker, D.C., McMahon Pratt, D., Butcher, J., Barreto, A.C. & Marsden, P.D., 1985. A focus of mucocutaneous leishmaniasis in Tres Bracos, Bahia, Brazil: characterization and identification of Leishmania stocks isolated from man and dogs. Transactions of the Royal Society of Tropical Medicine and Hygiene, 79:500-507.

Daoud, W. & Rageh, H.A., 1987. Analyse serologique, clinique et epidemiologique de 53 cas de kala-azar en Republique Arabe du Yemen. Bulletin de la Societe de Pathologie Exotique et de ses Filiales (Paris), 79:507-513.

Dedet, J.P. & Belazzoug, S., 1985. Leishmaniasis in North Africa. In: Leishmaniasis, (edited by Chang, K.P. & Bray, R.S.), Amsterdam, New York, Oxford, Elsevier, pp. 353-375.

Deibarra, A.A.L., Howard, J.G. & Sonary, D., 1982.

Monoclonal antibodies to Leishmania tropica major: specificities and antigen location. Parasitology, 85:523-531.

Dergacheva, T.I., Zherichina, I.I. & Rasnitsyna, N.M., 1979. A method of counting sandflies (Phlebotomidae). WHO/LEISH/79.15, WHO/VBC/79.718.

Draper, C.C. & Lillywhite, J.E., 1985. Immunodiagnosis of tropical parasitic infections. In: Recent Advances in Tropical Medicine, (edited by Gilles, H.M.), Churchill Livingstone, London, pp. 267-288.

Draper, C.C. & McLaren, M.L., 1981. Some applications of immunoassay in tropical parasitic infections. In: Immunoassays for the 80's, (edited by Voller, A., Bartlett, A. & Bidwell, D.E.), M.T.P. Press Ltd., London, pp. 449-456.

Edrissian, G.H., Darabian, P., Zovein, Z., Seyedi-Rashti, M.A. & Nadim, A., 1981. Application of the Indirect Fluorescent Antibody Test in the serodiagnosis of cutaneous and visceral leishmaniasis in Iran. Annals of Tropical Medicine and Parasitology, 75:19-24.

El-Adhami, B., 1976. Isolation of Leishmania from a black rat in the Baghdad area, Iraq. American Journal of Tropical Medicine and Hygiene, 25:759-761.

El-Amin, M.R., Wright, E.P., Kager, P.A., Laarman, J.J. & Pondman, K.W., 1985. ELISA using intact promastigotes for immunodiagnosis of kala-azar. Transactions of the Royal Society of Tropical Medicine and Hygiene, 79:344-350.

El-Amin, M.R., Wright, E.P., Abdel Rahman, A.M., Kolk, A., Laarman, J.J. & Pondman, K.W., 1986. Serodiagnosis of Sudanese visceral and mucosal leishmaniasis: comparison of ELISA - immunofluorescence and indirect haemagglutination. Transactions of the Royal Society of Tropical Medicine and Hygiene, 80:271-274.

El-Behairy, F., Jan, M.Y., Dhoparee, Z. & Omen, A., 1982. Haematologic and serologic aspects of visceral leishmaniasis in children in Saudi Arabia. Proceedings of the 7th Saudi Medical Meeting, Dammam, 3-6 May, 1982, King Faisal University, Dammam, pp. 228-245.

Elbihari, S., Kawasmeh, Z.A. & Al-Naiem, A.H., 1985. Possible reservoir host(s) of zoonotic cutaneous leishmaniasis in Al-Hassa Oasis, Saudi Arabia. Annals of Tropical Medicine and Parasitology, 78:543-545.

Elbihari, S., Kawasmeh, Z.A. & Al-Naiem, A. & Al-Atiya, S., 1987. Leishmania infecting man and animals in Saudi Arabia. 3. Leishmaniasis in Psammomys obesus, Cretzschmar in Al-Ahsa oasis, Saudi Arabia. Tropenmedizin und Parasitologie, 38:86-88.

Evans, D.A., 1987. Leishmania. In: In vitro methods for parasite cultivation, (edited by Taylor, A.E.R. & Baker, J.R.), Academic Press, pp. 52-75

Evans, D.A., Lanham, S.M., Baldwin, C.I. & Peters, W., 1984. The isolation and isoenzyme characterization of Leishmania braziliensis sub. sp. from patients with cutaneous

leishmaniasis acquired in Belize. Transactions of the Royal Society of Tropical Medicine and Hygiene, 78:35-42.

Evans, D.A. & Smith, V., 1986. A simple method for cloning leishmanial promastigotes. Zeitschrift fur Parasiten Kunde, 72:573-576.

Evencard, B., Linder, E. & Lundbergh, P., 1988. Standardization of a filter-paper technique for blood sampling. Annals of Tropical Medicine and Parasitology, 82:295-303.

Finnplanco-Ahmed & Hajjar, A., 1985. Existing conditions in Al-Baha Region. A report submitted to the Deputy Ministry for Town Planning, Ministry of Municipal and Rural Affairs, Kingdom of Saudi Arabia, Project No. 212, 2nd Report.

Fuller, G.K., Lemma, A., Haile, T. & Gemeda, N., 1979. Kala-azar in Ethiopia: survey of south-west Ethiopia. Annals of Tropical Medicine and Parasitology, 73:417-431.

Gardener, P.J., Chance, M.L. & Peters, W., 1974. Biochemical taxonomy of Leishmania. II. Electrophoretic variation of malate dehydrogenase. Annals of Tropical Medicine and Parasitology, 68:317-325.

Gardener, P.J. & Howells, R.E., 1972. Isoenzyme variation in leishmanial parasites. Journal of Protozoology, 19 (suppl.):47.

Garnham, P.C.C., 1971. The genus Leishmania. Bulletin of

the World Health Organization, 44:477-489.

Garnham, P.C.C., 1977. Global ecology of the leishmaniases. Introductory remarks. In: Ecologie des Leishmanioses, Colloques Internationaux du Centre National de la Recherche Scientifique, Paris, 239:2-3.

Gibson, W.C. & Miles, M.A., 1985. Application of new technologies to epidemiology. British Medical Bulletin, 41:115-121.

Githure, J.I., Oster, C.N. & Chulay, J.D., 1984. Comparison of three culture media for isolating Leishmania donovani from splenic aspirate. East African Medical Journal, 539.

Godfrey, D.G., 1984. Molecular biochemical characterization of human parasites. In: Recent Advances in Tropical Medicine, (edited by Gilles, H.M.), Churchill Livingstone Press, Edinburgh, London, Melbourne, New York, pp. 289-318.

Godfrey, D.G. & Kilqour, U.K., 1976. Enzyme electrophoresis in characterizing the causative organism of Gambian trypanosomiasis. Transactions of the Royal Society of Tropical Medicine and Hygiene, 70:219-224.

Gradoni, L., Pozio, E., Gramiccia, M., Maroli, M. & Bettini, S., 1983. Leishmaniasis in Tuscany (Italy). (VII). Studies on the role of the black rat, Rattus rattus, in the epidemiology of visceral leishmaniasis. Transactions of the Royal Society of Tropical Medicine and Hygiene, 77:427-431.

Gramiccia, M., Bettini, S., Gradoni, L. & Pozio, E., 1982.

Leishmania isolates from mammals in Italy. Acta Mediterranea di Patologia Infectiva e Tropicale, 1:103-108.

Haile, T. & Lemma, A., 1977. Isolation of Leishmania parasites from Arvicanthis in Ethiopa. Transactions of the Royal Society of Tropical Medicine and Hygiene, 71:180-181.

Harith, A.E., Kolk, A.H., Kager, P.A., Leeuwenburg, J., Muigai, R., Kiugu, S., Kiugu, S. & Laarman, J.J., 1986. A simple and economical direct agglutination test for serodiagnosis and sero-epidemiological studies of visceral leishmaniasis. Transactions of the Royal Society of Tropical Medicine and Hygiene, 32:943-946.

Harrison, D.L., 1968. The Mammals of Arabia. Vol. 2, Carnivora, Hydracoidea, Artiodactyla, Ernest Benn, London.

Harrison, D.L., 1972. The Mammals of Arabia. Vol. 3, Lagomorpha, Rodentia, Ernest Benn, London.

Hendrickes, L. & Wright, N., 1979. Diagnosis of cutaneous leishmaniasis by in vitro cultivation of saline aspirates in Schneider's Drosophila medium. American Journal of Tropical Medicine and Hygiene, 28:962-964.

Hommel, M., Peters, W., Ranque, J., Quilici, M. & Lanotte, G., 1978. The micro-ELISA technique in the serodiagnosis of visceral leishmaniasis. Annals of Tropical Medicine and Parasitology, 72:213-218.

Ho, M., Leeuwenburg, J., Mbugua, C., Wamachi, A. & Voller, A., 1983. An enzyme-linked immunosorbent assay (ELISA) for

field diagnosis of visceral leishmaniasis. American Journal of Tropical Medicine and Hygiene, 32:943-946.

Ho, M., Siongok, T.K., Lyerly, W.H. & Smith, D.H., 1982. Prevalence and disease spectrum in a new focus of visceral leishmaniasis in Kenya. Transactions of the Royal Society of Tropical Medicine and Hygiene, 76: 741-746.

Hoogstraal, H. & Heyneman, D., 1969. Leishmaniasis in the Sudan Republic. 30. Final epidemiologic report. American Journal of Tropical Medicine and Hygiene, 18:1091-1210.

Howard, J.G., Hale, C.H.R., Ling, W. & Chan-Liew, 1980. Immunological regulation of experimental cutaneous leishmaniasis. I. Immunogenetic aspect of susceptibility to Leishmania tropica in mice. Parasite Immunology, 2:303-314.

Jaffe, C. & McMahon-Pratt, D., 1983. Monoclonal antibodies specific for Leishmania tropica, 1. Characterization of antigens associated with stage- and species-specific determinants. The Journal of Medical Immunology, 131:1987-1993.

Kadhim, A.H., Sukkar, F.J., Al-Maghazachi, S.J. & Qawal, D., 1979. Evaluation of the role of foxes and jackals as a possible reservoir host for visceral leishmaniasis in central Iraq. Bulletin of the Biological Research Centre, 2:3-6.

Kellina, O.I., 1981. Problems and current lines in investigations on the epidemiology of leishmaniasis and its control in the USSR. Bulletin de la Societe de Pathologie

Exotique, 74:306-318.

Kellina, O.I. & Passova, O.M., 1985. A new leishmanial parasite of the Great Gerbil (Rhombomys opimus) in the USSR. Transactions of the Royal Society of Tropical Medicine and Hygiene, 79:872-874.

Kenzotange & Urtec, 1976. Southern Region Project Study, Deputy Ministry for Town Planning Affairs, Ministry of Municipal and Rural Affairs, Kingdom of Saudi Arabia.

Kilgour, V., Gardener, P.J., Godfrey, D.G. & Peters, W., 1974. Demonstration of electrophoretic variation of two amino transferases in Leishmania. Annals of Tropical Medicine and Parasitology, 68:245-264.

Kilgour, V. & Godfrey, D.G., 1973. Species characteristic isoenzymes of two amino transferases in trypanosomes. Nature, New Biology, 244: 69-70.

Killick-Kendrick, R., 1979. Biology of leishmania in phlebotomine sandflies. In: Biology of the Kinetoplastida, Vol. 2, (Eds. W.H.R.Lumsden & D.A.Evans), Academic Press: London / New York, pp 395-460.

Killick-Kendrick, R., 1987. Methods for the study of phlebotomine sandflies. In: The Leishmaniases in Biology and Medicine, Vol. 1., (edited by Peters, W. & Killick-Kendrick, R.), Academic Press, London, pp. 473-497.

Killick-Kendrick, R., Leaney, A.J., Peters, W., Rioux, J.A.

& Bray, R.S., 1985. Zoonotic cutaneous leishmaniasis in Saudi Arabia: the incrimination of Phlebotomus papatasi as the vector in the Al-Hassa Oasis. Transactions of the Royal Society of Tropical Medicine and Hygiene, 79:252-255.

Killick-Kendrick, R. & Molyneux, D.H., 1981. Transmission of leishmaniasis by the bite of phlebotomine sandflies: Possible mechanisms. Transactions of the Royal Society of Tropical Medicine and Hygiene, 75:152-154.

Killick-Kendrick, R. & Rioux, J.A., 1981. The Cevennes focus of leishmaniasis in southern France and the biology of the vector, Phlebotomus ariasi. In: Parasitological Topics, (edited by Canning, E.U.), Society of Protozoologists, Special Publication, 1:136-145.

Killick-Kendrick, R., Rioux, J.A., Bailly, M., Guy, M.W., Wilkes, T.J., Guy, F.M., Davidson, I., Knechtli, R., Ward, R.D., Guilvard, E., Perieres, J. & Dubois, H., 1984. Ecology of leishmaniasis in the south of France. 20. Dispersal of Phlebotomus ariasi Tonnoir, 1921, as a factor in the spread of visceral leishmaniasis in the Cevennes. Annales de Parasitologie Humaine et Comparee, 59:55-572.

Killick-Kendrick, R. & Ward, R.D., 1981. Workshop no. 11, Ecology of Leishmania. Parasitology, 82:143-152.

Kirk, R. & Lewis, D.J., 1946a. Taxonomy of the Ethiopian sandflies (Phlebotomus). I. Classification and synonymy. Annals of Tropical Medicine and Parasitology, 40:34-51.

Kirk, R. & Lewis, D.J., 1946b. Taxonomy of the Ethiopian

sandflies (Phlebotomus). II. Keys for the identification of the Ethiopian species. Annals of Tropical Medicine and Parasitology, 40:117-128.

Kirk, R. & Lewis, D.J., 1951. The phlebotominae of the Ethiopian Region. Transactions of the Royal Entomological Society of London, 102: 383-510.

Kirmse, P., Mahin, L. & Lahrech, T.M., 1987. Canine leishmaniasis in Morocco with special reference to infantile kala-azar. Transactions of the Royal Society of Tropical Medicine and Hygiene, 81:212-213.

Kozevnikov, P.V., 1963. Two nosological forms of cutaneous leishmaniasis. American Journal of Tropical Medicine and Hygiene, 12:719-724.

Lainson, R., Miles, M.A. & Shaw, J.J., 1981. On the identification of viscerotropic leishmanias. Annals of Tropical Medicine and Parasitology, 75:251-253.

Lainson, R. & Shaw, J.J., 1972. Leishmaniases of the New World: taxonomic problems. British Medical Bulletin, 28:44-48.

Lainson, R. & Shaw, J.J., 1979. The role of animals in the epidemiology of South American Leishmaniasis. In: Biology of Kinetoplastida, Vol. 2., (edited by Lumsden, W.H.R. & Evans, D.A.), Academic Press, London, New York, pp. 1-116.

Lainson, R. & Shaw, J.J., 1987. Evolution, classification and geographical distribution. In: The Leishmaniases in

Biology and Medicine, Vol.1., (edited by Peters, W. & Killick-Kendrick, R.), Academic Press, London, pp. 1-120.

Lane, R.P., 1986. The sandflies of Egypt (Diptera: Phlebotominae). Bulletin of the British Museum of Natural History (Entomology Series), 52:1-35.

Lane, R.P. & Al-Taqi, M., 1983. Sandflies (Diptera: Phlebotomidae) and leishmaniasis in Kuwait. Bulletin of Entomological Research, 73:633-644.

Lanham, S.M., 1982. Kits for isoenzyme characterization of Leishmania isolates in the field. In: Biochemical Characterization of Leishmania, (edited by Chance, M.L. & Walton, B.C.), Geneva: UNDP/ World Bank/WHO, pp. 87-95.

Lanotte, G., Rioux, J.A., Croset, H. & Vollhardt, Y., 1978. Ecologie des leishmaniose dans le sud de la France. Annales de Parasitologie, (Paris), 53:33-45.

Larsane, R.P., 1984. The zoogeographical composition and distribution of the Arabian butterflies (Lepidoptera: Rhopalocera). Journal of Biogeography, 5:119-158.

Le Blancq, S.M., Lanham, S.M. & Evans, D.A., 1987. Comparative isoenzyme profiles of Old and New World Leishmania. In: The Leishmaniases in Biology and Medicine, Vol. 1. (edited by Peters, W. & Killick-Kendrick, R.), Academic Press, London, pp. 543-550.

Le Blancq, S.M. & Peters, W., 1986a. Leishmania in the Old World. 4. The distribution of Leishmania donovani sensu lato

zymodemes. Transactions of the Royal Society of Tropical Medicine and Hygiene, 80:367-377.

Le Blancq, S.M. & Peters, W., 1986b. Leishmania in the Old World. 2. Heterogeneity among Leishmania tropica zymodemes. Transactions of the Royal Society of Tropical Medicine and Hygiene, 80:113-119.

Le Blancq, S.M., Schnur, L.F. & Peters, W., 1986. Leishmania in the Old World. 1. The geographical and hostal distribution of L. major zymodemes. Transactions of the Royal Society of Tropical Medicine and Hygiene, 80:99-112.

Lewis, D.J., 1982. A taxonomic review of the genus Phlebotomus (Diptera: Psychodidae). Bulletin of the British Museum (Natural History), 45:121-209.

Lewis, D.J. & Buttiker, W., 1980. Insects of Saudi Arabia. Diptera: Fam. Psychodidae, Subfam. Phlebotominae. Fauna of Saudi Arabia, 2:252-285.

Lewis, D.J. & Buttiker, W., 1982. Insects of Saudi Arabia.

The taxonomy and distribution of Saudi Arabian phlebotomine sandflies (Diptera:Psychodidae). Fauna of Saudi Arabia, 4:353-397.

Lewis, D.J. & Buttiker, W., 1986. Some phlebotomine sandflies (Diptera:Psychodidae) from Saudi Arabia. Fauna of Saudi Arabia, 8:324-339.

Lewis, D.J. & Ward, R.D., 1987. Transmission and vectors. In: The Leishmaniases in Biology and Medicine, Vol. 1.,

(Edited by Peters, W. & Killick-Kendrick, R.), Academic Press, London, pp. 235-251.

Lightner, L.K., Chulay, J.D. & Bryceson, A.D.M., 1983. Comparison of microscopy and culture in the detection of Leishmania donovani from splenic aspirates. American Journal of Tropical Medicine and Hygiene, 32:296-299.

Lysenko, A.J., 1971. Distribution of leishmaniasis in the Old World. Bulletin of the World Health Organization, 44:515-520.

Mahmoud, A.A., Al-Tuwaijri, A., Al-Mofleh, I. & Al-Khuwaitir, S.A., 1985. In vitro and in vivo isolation of Leishmania tropica from Saudi Arabia. Parasitology Research, 71:271-272.

Manson-Bahr, P.E.C., 1984. Leishmaniasis. (b) Cutaneous leishmaniasis. In: Recent Advances in Tropical Medicine, (edited by Gilles, H.M.), Churchill Livingstone Press, Edinburgh, London, Melbourne, New York, pp. 88-102.

Mansory, M.A., 1977. Periodic market in the southwest region of Saudi Arabia: A study in human geography. M.A Thesis, Michigan State University, pp. 162.

Mansueto, S., Miceli, M.D. & Quartararo, P., 1982. Counter-immunoelectrophoresis (CIEP) and ELISA tests in the diagnosis of canine leishmaniasis. Annals of Tropical Medicine and Parasitology, 76:229-231.

Marinkelle, C.J., 1980. Assignment report. Leishmaniasis in

Saudi Arabia. 17 March 1980-12 May 1980. Cyclostyled report EM/PD/12 EM/CD/24 EM/SAA/MPD/003, World Health Organization, Geneva, pp. 1-13, annexes I and II.

Marinkelle, C.J., 1985a. Visceral leishmaniasis with special reference to Saudi Arabia: diagnosis and treatment. In: Medical Symposium on Leishmaniasis, March 22-24, 1980, Dammam, Ministry of Health, Riyadh, pp. 107-110.

Marinkelle, C.J., 1985b. Epidemiology of cutaneous leishmaniasis with special reference to Saudi Arabia. In: Medical Symposium on Leishmaniasis, March 22-24, 1980, Dammam, Ministry of Health, Riyadh, pp. 137-146.

McMahon-Pratt, D. & David, J.R., 1982. Applications of monoclonal antibodies specific for Leishmania species. In: Biochemical Characterization of Leishmania, (edited by Chance, M.L. & Walton, B.C.), Geneva, UNDP/World Bank/WHO, pp. 248-257.

Melo, N.M., de Azevedo, H.P., Rohman, I. & Mayrink, W., 1985. A new defined medium for cultivating leishmaniasis promastigotes. Acta Tropica, 42:137-141.

Menzel, S. & Bienzle, U., 1978. Antibody responses in patients with cutaneous leishmaniasis of the Old World. Tropenmedizin und Parasitologie, 29:194-197.

Michael, S.A., Morsy, T.A., Abou El-Seoud, S.F. & Saleh, M.S.A., 1986. Leishmaniasis antibodies in stray cats in Ismailiya Governate, Egypt. Information Circular, WHO mediterranean Zoonoses Control Centre, 2:1-2.

Migahid, A.A. & Hammouda, S., 1978. Flora of Saudi Arabia. Second Edition, Riyadh University Publication, 1:1-647.

Miles, A.M., 1980. Leishmania - culture and biochemical comparisons. Some difficulties. Proceedings of a workshop held at the Pan American Health Organization, Washington D.C., 9-11 December, 1980. UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, pp. 123-137.

Miles, A.M., 1985. Biochemical identification of the leishmanias. PAHO Bulletin, 4:343-353.

Minter, D.M., Wijers, D.J.B., Heisch, R.B. & Manson-Bahr, P.E.C., 1962. Phlebotomus martini - a probable vector of kala-azar in Kenya. British Medical Journal, 2:835.

Molyneux, D.H. & Ashford, R.W., 1983. The Biology of Trypanosoma and Leishmania. Parasites of Man and Domestic Animals. Taylor and Francis, London, pp. 294.

Morsy, T.A. & Mawwary, C.H., 1974. Leishmanin skin tests in Riyadh, Saudi Arabia. Journal of the Kuwait Medical Association, 8:168-171.

Morsy, T.A., Michael, S.A. & El Disi, A.M., 1980. Cats as reservoir hosts of human parasites in Amman, Jordan. Journal of the Egyptian Society of Parasitology, 10:5-18.

Morsy, T.A. & Shoura, M.I., 1976. Some aspects of cutaneous leishmaniasis in Riyadh, Saudi Arabia. Journal of Tropical Medicine and Hygiene, 79:137-139.

Mukerji, K., Roy, S., Mukhopadhyay, P., Gupta, P.K. & Ghosh, D.K., 1984. Evaluation of different subcellular fractions of Leishmania donovani for immunodiagnosis of visceral leishmaniasis. Indian Journal of Experimental Biology, 22:120-122.

Mutinga, M.J., Ngoka, J.M., Schnur, L.F. & Chance, M.L., 1980. The isolation of leishmanial parasites from domestic dogs in Machakos District of Kenya, and the possible role of dogs as reservoirs of kala-azar in East Africa. Annals of Tropical Medicine and Parasitology, 74:139-144.

Nadim, A., Javadian, E., Noushin, M.K. & Nayil, A.K., 1979a. Epidemiology of cutaneous leishmaniasis in Afghanistan. Part 1. Zoonotic cutaneous leishmaniasis. Bulletin de la Societe de Pathologie Exotique, 72:31-35.

Nadim, A., Javadian, E., Noushin, M.K. & Nayil, A.K., 1979b. Epidemiology of cutaneous leishmaniasis in Afghanistan. Part 2. Anthroponotic cutaneous leishmaniasis. Bulletin de la Societe de Pathologie Exotique, 72:461-466.

Nadim, A., Seyedi-Rashti, M.A. & Ashi, J., 1979c. Cutaneous leishmaniasis in Saudi Arabia: An overview. Bulletin de la Societe de Pathologie Exotique, 72:237-244.

Neranov, V.M., Malkhazonova, S.M. & Tikunov, V.S., 1986. Experience in dividing up the nosogeographic range of cutaneous leishmaniasis in the old world. Medical Protozoology, 2: 49-55 (English summary).

Newton, B.A., 1976. Biochemical approaches to the taxonomy

of kinetoplastid flagellates. In: Biology of the Kinetoplastida, (edited by Lumsden, W.H.R. & Evans, D.A.), Academic Press, London, New York, San Francisco, Vol. 1., pp. 405-434.

Ngoka, J.M. & Mutinga, M.J., 1977. The dog as a reservoir of visceral leishmaniasis in Kenya. Transactions of the Royal Society of Tropical Medicine and Hygiene, 71:447-448.

Niazi, A.D., 1980. Studies in the epidemiology and seroepidemiology of visceral leishmaniasis in Iraq. PhD Thesis, University of London, pp. 135-308.

Nicolle, C.H. & Comte, C.H., 1908. Recherches sur le kala-azar entreprises a l'Institut Pasteur de Tunis. IV. Canine reservoirs du kala-azar. Achives de L'Institut Pasteur de Tunis, 3:59-62.

Pampiglioni, S., Manson-Bahr, P.E.C., Giungi, F., Giunti, G., Parenti, A. & Canestri-Trotti, G., 1974. Studies on mediterranean leishmaniasis. 2. Asymptomatic cases of visceral leishmaniasis. Transactions of the Royal Society of Tropical Medicine and Hygiene, 68:447-453.

Pappas, M.G., Hajkowski, R. & Hockmeyer, W.T., 1983a. Dot Enzyme-Linked Immunosorbent Assay (DOT-ELISA): A micro technique for the rapid diagnosis of visceral leishmaniasis. Journal of Immunological Methods, 64:205-214.

Pappas, M.G., Hajkowski, R., Diggs, C.L. & Hockmeyer, W.T., 1983b. Disposable nitrocellulose filtration plates simplify the Dot-ELISA for serodiagnosis of visceral leishmaniasis.

Transactions of the Royal Society of Tropical Medicine and Hygiene, 79:136

Pappas, M.G., Hajkowski, R. & Hockmeyer, W.T., 1984. Standardization of the Dot Enzyme-Linked Immunosorbent Assay (DOT-ELISA) for human visceral leishmaniasis. American Journal of Tropical Medicine and Hygiene, 33:1105-1111.

Perfil'ev, P.P., 1966. Phlebotomidae (sandflies). Fauna of U.S.S.R. Diptera. Translated by the Israel Programme for Scientific Translations, from 1966 original (Academy of Science, USSR, Diptera, 3, New Series No. 93), pp. 363.

Peters, W., 1985a. The leishmaniases - the parasites and the host's response to infection. In: Medical Symposium on Leishmaniasis, March 22-24, 1980, Dammam, Ministry of Health, Riyadh, pp. 11-29.

Peters, W., 1985b. Diagnostic procedures in leishmaniasis. In: Medical Symposium on Leishmaniasis, March 22-24, 1980, Dammam, Ministry of Health, Riyadh, pp. 30-56.

Peters, W. & Al-Zahrani, M.A., 1987. The leishmaniases - a public health problem in Saudi Arabia. Saudi Medical Journal, 8:333-343.

Peters, W., Elbihari, S. & Evans, D.A., 1986. Leishmania infecting man and wild animals in Saudi Arabia. 2. Leishmania arabica n. sp. Transactions of the Royal Society of Tropical Medicine and Hygiene, 80:497-502.

Peters, W., Elbihari, S., Liu, C. Le Blanco, S.M., Evans,

D.A., Killick-Kendrick, R., Smith, V. & Baldwin, C.I., 1985. Leishmania infecting man and wild animals in Saudi Arabia. 1. General Survey. Transactions of the Royal Society of Tropical Medicine and Hygiene, 79:831-839.

Peters, W., Evans, D.A. & Lanham, S.M., 1983. Importance of parasite identification in cases of leishmaniasis. Journal of the Royal Society of Medicine, 76:540-542.

Petrisceva, P.A., 1971. The natural focality of leishmaniasis in the USSR. Bulletin of the World Health Organization, 44:567-576.

Phillips, L., 1904. Note on the occurrence of the Leishman-Donovan parasite in Arabia and Egypt. The Journal of Tropical Medicine, 7:236-237.

Pozio, E., Gradoni, L., Bettini, S. & Gramiccia, M., 1981. Leishmaniasis in Tuscany (Italy). VI. Canine leishmaniasis in the focus of Monte Argentario (Grosseto). Acta Tropica, 38:383-393.

Prasad, L.S., Saran, R. & Sells, P., 1980. Microplate Enzyme Linked Immunosorbent Assay for visceral leishmaniasis. Indian Journal of Medical Research, 71:708-711.

Pringle, G., 1956. Kala-azar in Iraq: preliminary epidemiological considerations. Bulletin of Endemic Diseases (Baghdad), 1:275-294.

Pringle, G., 1957. Oriental sore in Iraq: historical and

epidemiological problems. Bulletin of Endemic Diseases (Baghdad), 2:41-76.

Ranque, P., 1977. Les leishmanioses au Senegal. Etude epidemiologique et ecologique. In: Ecologie des Leishmanioses, Colloques Internationaux due Centre National de la Recherche Scientifique, Paris, 239: 225-232.

Rassam, M.B. & Al-Mudhaffer, S.A., 1979. The primary isolation of Leishmania donovani from Iraq on different culture media. Annals of Tropical Medicine and Parasitology, 73:345-347.

Rassam, M.B. & Al-Mudhaffer, S.A., 1980. Comparative diagnostic study of kala-azar. Annals of Tropical Medicine and Parasitology, 74:283-287.

Reed, S.M., Badaro, R., Masur, H., Carvalho, E.M., Lorenco, R., Lisboa, A., Teixeira, R., Johnson, W.D. & Jones, T.C., 1986. Selection of a skin test antigen for American visceral leishmaniasis. American Journal of Tropical Medicine and Hygiene, 35:79-85.

Rioux, J.A., Daoud, W., Pratlong, F., El Kubati, Y., Morenco, G., Rageh, H.A., Brun, R., Mouharem, A., Martinez Ortega, E. & Belmonte, A., 1986. Les complexes Leishmania donovani s. st. Leishmania tropica et Leishmania major en Republique Arabe du Yemen. In: Leishmania. Taxonomie et phylogenese. Applications eco-epidemiologiques. (Colloques internationals CNRS/INSERM, 1984). IMEEE, Montpellier, pp. 357-363.

Rioux, J.A., Lanotte, G., Pratlong, F., Dereure, J., Jarry, D., Moreno, G., Killick-Kendrick, R., Perieres, J., Guilvard, E., Belmonte, A. & Portus, M., 1985. La leishmaniose cutanee autochtone dans le sud-est de la France. Resultats d'une enquete eco-epidemiologique dans les Pyrenees-Orientales. Medecine et Maladies Infectieuses, 11:650-656.

Saf'janova, V.M., 1971. Leishmaniasis control. Bulletin of the World Health Organization, 44:561-566.

Schlein, Y., Warburg, A., Schnur, L.F., Le Blancq, S.M. & Gunders, A.E., 1984. Leishmaniasis in Israel: reservoir hosts, sandfly vectors and leishmanial strains in the Negev, Central Arava, and along the Dead Sea. Transactions of the Royal Society of Tropical Medicine and Hygiene, 78:480-484.

Schnur, L.F., Chance, M.L., Ebert, F., Thomas, S.C. & Peters, W., 1981. The biochemical and serological taxonomy of visceralizing Leishmania. Annals of Tropical Medicine and Parasitology, 75:131-144.

Sebai, Z.A. & Morsy, T.A., 1976. Cutaneous leishmaniasis in Bisha Town, Saudi Arabia. Journal of Tropical Medicine and Hygiene, 79:89-91.

Sells, P.G. & Burton, M., 1981. Identification of Leishmania amastigotes and their antigens in formalin fixed tissue by immunoperoxidase staining. Transactions of the Royal Society of Tropical Medicine and Hygiene, 75:461-468.

Sheriff, D., 1957. Canine visceral leishmaniasis in

foxhounds near Baghdad. Transactions of the Royal Society of Tropical Medicine and Hygiene, 51:467.

Smith, D.H., 1984. Leishmaniasis. (a) visceral leishmaniasis - human aspects. In: Recent Advances in Tropical Medicine, (edited by Gilles, H.M.), Churchill Livingston Press, Edinburgh, London, Melbourne, New York, pp. 79-87.

Smithies, O., 1955. Zone electrophoresis in starch gels, group variations in the serum protein of normal human adults. Biochemical Journal, 61: 629-641.

Southgate, B.A., 1977. The structure of foci of visceral leishmaniasis in north-eastern Kenya. In: Ecologie des Leishmanioses, Colloques Internationaux due Centre National de la Recherche Scientifique, Paris, 239: 241-247.

Southgate, B.A. & Oriedo, V.E., 1962. Studies in the epidemiology of East African leishmaniasis. 1. The circumstantial epidemiology of kala-azar in the Kitui District of Kenya. Transactions of the Royal Society of Tropical Medicine and Hygiene, 56:30-47.

Srivastava, L., Chakravarty, A.K., Kumar, A. & Pradeep, K., 1984. Comparison of ELISA and indirect immunofluorescence in sero-epidemiology of kala-azar. Indian Journal of Medical Research, 79:744-748.

Steiger, R.F. & Steiger, E. (1977). Cultivation of Leishmania donovani and Leishmania braziliensis in defined media: nutritional requirements. Journal of Protozoology,

24:437-441.

Sukkar, F., 1978. Kala-azar in Iraq in 1975. Bulletin of Endemic Diseases (Baghdad), 19:29-38.

Sukkar, F., 1985. Leishmaniasis in the Middle East. In: Leishmaniasis (edited by Chang, K.P. & Bray, R.S.), Elsevier, Amsterdam, New York, Oxford, pp. 393-413.

Sukkar, F., Al-Mahdawi, S.K., Al-Doori, N.A. & Kadhum, J.A., 1981. Isolation of Leishmania from the spleen of a dog in Iraq Transactions of the Royal Society of Tropical Medicine and Hygiene, 75:859-860.

Tarizzo, M.L., Bracken, H.A., & Strait, D.J., 1953. A case of visceral leishmaniasis in Saudi Arabia. American Journal of Tropical Medicine and Hygiene, 2:846-849.

Taylor, A.E.R. & Baker, J.R., 1978. Methods of cultivating parasites in vitro. Academic Press, London & New York.

Taylor, A.E.R. & Baker, J.R., 1968. The cultivation of parasites in vitro. Blackwell Scientific Publications, Oxford.

Theodor, O., 1948. Classification of the Old World species of the subfamily Phlebotominae (Diptera: Psychodidae). Bulletin of Entomological Research, 39:85-115.

Theodor, O., 1958. Psychodidae-phlebotominae. Die Fleigender Palaearktischen Region, 90:1-55.

Tobie, E.J., Von-Brandt, T. & Mehlman, B., 1950. Cultural and physiological observations on Trypanosoma rhodesiensis and Trypanosoma gambiense. Journal of Parasitology, 36:48-54.

Trager, W., 1957. Nutrition of haemo-flagellate Leishmania tarentolae having an interchangeable requirement for choline or pyridoxal. Journal of Protozoology, 4:269-276.

Voller, A., Bartlett, A. & Bidwell, D.E., 1976. Enzyme immunoassays for parasitic diseases. Transactions of the Royal Society of Tropical Medicine and Hygiene, 70: 98-106.

Voller, A., Bidwell, D.E. & Bartlett, A., 1980. Enzyme linked immunosorbent assay. In: Manual of Clinical Immunology, (edited by Rose, N. & Friedman, H.), Washington D.C., American Society of Microbiology, pp. 359-371.

Werner, G.T., Frosner, G.G. & Epp. C. (1985). Capillary blood on dried spots has only limited value for sero-epidemiological research. Transactions of the Royal Society of Tropical Medicine and Hygiene, 79:135.

Woodruff, A.W., 1973. Mechanisms involved in anaemia associated with infection and splenomegaly in the tropics. Transactions of the Royal Society of Tropical Medicine and Hygiene, 67:313-328.

World Health Organization, 1984. The leishmaniases. Technical Report Series, No. 701, Geneva.

Wraxall, B.G.D. & Gilliford, B.J., 1968. A thin starch gel

method for enzyme typing of blood stains. Journal of the Forensic Science Society, 8:81-82.

Zeese, W. & Frank, W., 1987. Present epidemiological situation of kala-azar in the Republic of Sudan. Zentralblatt fur Bakteriologie, Mikrobiologie und Hygiene, 264:414-421.

Zeldon, R. & Ponce, C., 1974. Parasitological and immunological diagnosis of cutaneous leishmaniasis in the New World. Proceedings of the 3rd International Congress of Parasitology, Munich, Federal Republic of Germany, 25-31 August, 1974, 1:239-240.

ANNEX 1

EPIDEMIOLOGICAL SURVEY FORM FOR A CASE OF VISCERAL LEISHMANIASIS

١) اسم المريض (رباعيا) :

1) Name of the Patient (Full)

2) Sex: Male Female

٢) الجنس : ذكر انثى

3) Age:

٣) العمر :

4) Nationality:

٤) الجنسية :

5) Occupation:

٥) العمل :

٦) العنوان(محل الاقامة بالتفصيل) : اسم القرية اسم شيخ القبيلة اسم الامارة :

6) Residence (Full Address):

Name of Village Chief of the Tribe Amarah

٧) تاريخ القدوم للمنطقة

للملكة (للوافدين)

7) Date of Entry to - Region

- Kingdom (Non-Saudis)

٨) تحركات المريض :

....

....

8) Movements of the patient:

....

....

٩) تاريخ بداية ظهور اعراض المرض ومكانه :

9) Place and date of appearance of symptoms:

١٠) مكان الاصابة المحتمل :

10) Probable place of infection:

١١) التشخيص المبدئي :

11) Provisional Diagnosis:

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ANNEX 1 cont.

12) Patient has been: Hospital
seen in : Primary Health Care Centre

مستشفى
مركز صحى

١٢) تم فحص المريض بمستشفى او مركز صحى :

13) Date of Examination:

/ / 140
/ / 19

١٣) تاريخ الفحص : / / ١٤٠ هـ
/ / ١٩ م

14) Patient is Diagnosed by: Clinically - Laboratory - Both

١٤) تم تشخيص المريض : اكلينيكيا مخبريا بهما معا

15) In case of laboratory Diagnosis mention the Test:

١٥) اذا كان التشخيص مخبريا ما هو نوع الاختبار :

16) Name of Physicians:

١٦) اسم الطبيب المعالج :

17) Date of Admission:

١٧) تاريخ دخول المستشفى :

18) Date of Discharge:

١٨) تاريخ الخروج من المستشفى :

19) Treatment given:

١٩) نوع العلاج :

20) No of doses:

٢٠) عدد الجرعات التى اعطيت :

٢١) نتيجة الفحص بعد العلاج: سلبى ايجابى لم تعمل

21) Result of Examination after treatment:
Negative - Positive - Not done

٢٢) هل لاحظ الطبيب او الباحث وجود اى تقرحات جلدية فى المريض : نعم – لا .

22) Did the Doctor or investigator notice any cutaneous
lesion in the patient: Yes - No

٢٣) أ – عدد غرف السكن :

ب – نوع السكن :

23) A / No of rooms:

B / Type of house:

C / Are there wire nets around the doors and windows:

جـ – هل توجد شباك سلك دقيقة الفتحات حول النوافذ و الابواب :

D / Does the patient use mosquito net during sleeping:

د – هل يستعمل المريض ناموسية اثناء النوم :

E / Patient used to sleep: Outside - Inside

هـ) المريض اعتاد النوم : داخل المنزل خارج المنزل

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٢٤) بيان بالمخالطين المباشرين بمنزل المريض والتحرى عن وجود اصابات مماثلة
جدول رقم (١) .

24) No of direct contacts with the patient and positive cases among them; table (1)

٢٥) عدد عينات الدم التى تم سحبها من المخالطين ونتيجة فحصها
جدول رقم (١) .

25) No of collected blood samples from the contacts & test results Table (1)

٢٦) ما هى الحيوانات التى لاحظ المريض أو احد افراد عائلته او الباحث
وجودها مع تسجيلها بالترتيب حسب كثافتها .

١) ٢) ٣)

26) Which are the most prevalent animals noticed near the patients' houses
(1) (2) (3)

٢٧) هل لاحظت فى الحيوانات الموضحة اعلاه وجود اى تقرحات :

نعم لا

27) Have you seen any lesions in the Animals which you noticed:
Yes No

٢٨) عدد المصائد التى تم وضعها فى منزل المريض :

لاصقة ضوئية

28) No of traps: Sticky Trap:
Light Trap:

٢٩) عدد الحشرات ونوعها ونتيجة فحصها وتشريحها حسب ما هو موضح بالجدول رقم (٢) .

29) No of collected Sandflies, classification and Dissection as shown in Table (2)

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ANNEX 1 cont.

الاستـنـتـاج :

SUMMARY OF FINAL RESULTS:

المريض تم تشخيصه فى بواسطة الدكتور

1) Patient has been diagnosed in by Physician

٢) دخول المريض المستشفى فى وخرج فى
بعد اعطائه العلاج اللازم والتأكد من سلبية الفحص بعد العلاج اذا كان غير ذلك
اذكره فى الملاحظات .

2) Date of Admission

Date of Discharge (After cure of the patient which should be confirmed by laboratory if not mentioned in the remarks)

٣) الحيوانات التى لوحظ وجودها بكثرة هى حسب كثافة وجودها كما يلى :

١) ٢) ٣) ٤)

3) Animals most noticed are:

(1) (2) (3) (4)

٤) الحشرات التى تم جمعها هى : داخل ذكر انثى
خارج ذكر انثى

4) Collected sandflies: Outdoors - Male
Female
Indoors - Male
Female

٥) تم تشريح عدد عينة كانت سلبى ايجابى

5) Dissected sandflies - Number
Positive
Negative

٦) تم رش المنزل بمبيد :

6) Spraying done by insecticide:

٧) الملاحظات :

7) Remarks:

(1) ١)
(2) ٢)
(3) ٣)

Name of Investigator: اسم الباحث :

Hospital Director: مدير المستشفى :

Director of Leishmania Center: 384 : مدير مركز مكافحة الليشمانيا

ANNEX 1 cont. TABLE 1

DIRECT CONTACT OF THE PATIENT

جدول رقم (١)

بيان بامخالطين المباشرين بمنزل المريض .

No. رقم	Name الاسم	Age عمر	Sex جنس	Nationality الجنسية	Date تاريخ	Blood Samples Collected and Results عينات الدم ونتيجة فحصها	Remarks ملاحظات

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ANNEX 1 cont.

TABLE NO.2

ENTOMOLOGICAL COLLECTION
FROM THE PATIENT RESIDANCY

جدول رقم (٢)

النشاطات الحشرية لعملية الاستقصاء الوبائي للمريض / ....

Classification الانواع (الحشرة)				Total of Collected Sand Flies مجموع العينات		No of Trap عدد المصائد		Place of Collec-tion مكان الجمع	Date التاريخ	No رقم
						Sticky	Light			

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ANNEX 2 Positive primary isolate cultures from cutaneous leishmaniasis patients from southwest Saudi Arabia

Culture No.	Age (years)	Patient Nationality	Lesion Site	Lesion Type	Duration (months)	Geographical Area (district)	Village	
A25	5	Saudi	arm	N/U	-	Abha	Kaldeh	(H)
A26	4	Saudi	shoulder	N/U	5	Abha	Shaar	(H)
A27	5	Saudi	arm	U	2	Bellahmer	Al-Mawaen	(H)
A29	7	Saudi	cheek	N/U	2	Abha	Ben-Malik	(H)
A32	5	Saudi	cheek	N	-	Bellahmer	Al-Mawaen	(H)
A35	54	Saudi	cheek	N/U	-	Abha	Abha	(H)
A36	30	Saudi	lip	N/U	2	Mahayel	Mahayel	(L)
A37	16	Saudi	cheek	N/U	-	Mahayel	Mahayel	(L)
A38	0.8	Saudi	cheek	N/U	-	Madentsultan	Hajla	(H)
A39	0.6	Saudi	nose	N	-	Khamis-Mushayt	Khamis-Mushayt	(H)
A40	50	Saudi	forearm	N/U	24	Abha	Abha	(H)
A41	27	Pakistani	nose	N	18	Shaaf Shahran	Al-Waddeen	(H)
A42	12	Saudi	forearm	N	1	Abha	Abha	(H)
A43	12	Saudi	cheek	N/U	1	Abha	Rabaet-Rufaedah	(H)
A46	40	Pakistani	nose	U	11	Abha	Banirezam	(H)
A48	13	Jordanian	forearm	N/U	5	Abha	Abha	(H)
A50	36	Saudi	arm	-	-	Abha	Abha	(H)
A51	22	Yemeni	arm	N/U	-	Abha	Abha	(H)
A53	-	-	-	-	-	-	-	-
A54	-	-	-	-	-	-	-	-
A56	-	Saudi	forearm	N/U	3	Bellahmer	Bellahmer	(H)
A59	13	Saudi	leg	N/U	2	Tath-Lith	Tath-Lith	(D)
A60	11	Saudi	cheek	-	2	Abha	Abha	(H)

Notes:
(A): Asir Province (B): Baha Province
(H): Highlands (L): Lowlands
(T): After treatment (D): Desert area to the east of the Asir plate

ANNEX 2 (continued) Positive primary isolate cultures from cutaneous leishmaniasis patients from southwest Saudi Arabia

Culture No.	Age (years)	Patient Nationality	Site	Lesion Type	Duration (months)	Geographical Area (district)	Village	
A62	12	Saudi	nose	N/U	4	Bellahmer	Bellahmer	(H)
A63	27	Saudi	arm	N	-	Abha	Abha	(H,T)
A65	28	Yemeni	lip	U	8	Kamis-Mushat	-	(H,T)
A67	14	Saudi	nose	-	-	Bellahmer	Abel	(H)
A68	19	Saudi	hand	N/U	1	Abha	Bani-Rezam	(H)
A69	29	Saudi	leg	U	0.7	Abha	Abha	(H)
A71	8	Saudi	lip	N	1	Abha	Abha	(H)
A72	42	Egyptian	cheek	N	1	Abha	Abha	(H)
A73	4	Saudi	chin	N/U	3	Bellahmer	Abel	(H)
A74	24	Pakistani	lip	N/U	3	Khamis-Mushayt	Khamis-Mushayt	(H)
A76	50	Saudi	thumb	-	4	Abha	-	(H)
A77	11	Saudi	ear	N	2	Sorat-Abidah	Sorat-Abidah	(H)
A78	17	Saudi	hand	U	1	Abha	Bani-Malik	(H)
A87	12	Yemeni	arm	N	0.3	Abha	Abha	(H)
A90	20	Saudi	-	N/U	3	Bellahmer	Al-Mawaen	(H)
A91	9	Saudi	lip	N/U	0.3	Abha	Bani-Malik	(H)
A93	8	Yemeni	cheek	N/U	2	Abha	-	-
A94	15	Saudi	nose	U	4	Abha	Al-Gara	(H)
A95	33	Egyptian	forehead	N	4	Abha	Bani-Rezam	(H)
A96	10	Saudi	lip	N/U	0.5	Abha	Abha	(H)
A97	57	Saudi	cheek	N/U	2	Abha	Al-Gara	(H)
A98	14	Saudi	cheek	N/U	6	Bil-Asmar	Bil-Asmar	(H)
A99	12	Saudi	nose	N/U	2	Abha	Abha	(H)

Notes:
(A): Asir Province
(H): Highlands
(T): After treatment
(B): Baha Province
(L): Lowlands
(D): Desert area to the east of the Asir plate

ANNEX 2 (continued) Positive primary isolate cultures from cutaneous leishmaniasis patients from southwest Saudi Arabia

Culture No.	Patient Age (years)	Patient Nationality	Lesion Site	Lesion Type	Lesion Duration (months)	Geographical Area (district)	Geographical Area Village	
A105	25	Yemeni	hand	scar	-	Abha	Abha	(H)
A106	2	Saudi	cheek	N/U	2	Abha	Abha	(H)
A109	40	Saudi	leg	N/U	3	Abha	Al-Gara	(H)
A111	10	Saudi	lip	N/U	-	Abha	Abha	(H)
A113	11	Saudi	cheek	N/U	4	Abha	Rabaet-Rufaedah	(H)
A114	15	Saudi	hand	N/U	2	Abha	Abha	(H)
A115	60	Saudi	nose	U	5	Al-Namis	Bani-Omar	(H)
A116	11	Saudi	cheek	U	1	Abha	-	(H)
A117	-	-	-	-	-	-	-	-
A119	-	Saudi	-	-	-	Mahayel	Mahayel	(L)
A122	-	-	-	-	-	Mahayel	Mahayel	(L)
A123	32	Thai	nose	U	5	Abha	-	(H)
A124	12	Saudi	-	N/U	5	Abha	Tymnah	(H)
A125	10	Saudi	-	N/U	2	Sorat-Abidah	Sorat-Abidah	(H)
A126	35	Saudi	-	N/U	2	Sorat-Abidah	Sorat-Abidah	(H)
A127	5	Saudi	-	N/U	2	Abha	Bani-Malik	(H)
A129	10	Saudi	nose	N	2	Gizan	Bal-Ghazi	(L)
A130	10	Saudi	nose	N	6	Rijal-Alma	Rijal-Alma	(L)
A131	22	Saudi	lip	N	2	Rijal-Alma	Rijal-Alma	(L)
A133	24	Sudanese	-	-	5	Abha	Abha	(H)
A134	4	Saudi	hand	N/U	-	Abha	Rabaet-Rufaedah	(H)
A135	27	Yemeni	lip	N/U	4	Abha	Akabt-Dela	(L,T)
A136	5	Saudi	cheek	N/U	2	Abha	Tymnah	(H)

Notes:
(A): Asir Province (B): Baha Province
(H): Highlands (L): Lowlands
(T): After treatment (D): Desert area to the east of the Asir plate

ANNEX 2 (continued) Positive primary isolate cultures from cutaneous leishmaniasis patients from southwest Saudi Arabia

Culture No.	Patient Age (years)	Nationality	Lesion Site	Type	Duration (months)	Geographical Area (district)	Village	
A137	10	Saudi	cheek	-	2	Abha	Abha	(H)
A138	35	Egyptian	lip	N/U	-	Abha	Al-Kam	(H)
B1	3	Saudi	cheek	-	-	Baha	Bel-Jurshe	(H)
B2	-	-	-	-	-	-	-	-
B3	1.5	Saudi	cheek	N/U	2	Dos	Dos-Bani-Ali	(H)
B5	-	-	-	-	-	-	-	-
B11	35	Saudi	arm	U	1	Al-Atawlah	Al-Atawlah	(H)
B13	2	Saudi	cheek	U	7	Ban-Hassan	Wadi-Asader	(H)
B15	9	Saudi	nose	-	5	Dos	Dos-Al-Namah	(H)
B16	6	Syrian	lip	-	2	Al-Atawlah	Al-Atawlah	(H)
B17	14	Saudi	lip	-	1	Baha	Al-Mosa	(H)
B635	35	Saudi	cheek	-	2	Bani-Kaber	Al-Hadab	(H)
B503	-	-	-	-	-	Baha	-	(H)
B849	-	-	-	-	-	Baha	-	(H)
B848	-	-	-	-	-	Baha	-	(H)
B812	-	-	-	-	-	Baha	-	(H)
B956	-	-	-	-	-	Baha	-	(H)

Notes:

(A): Asir Province (B): Baha Province
(H): Highlands (L): Lowlands
(T): After treatment (D): Desert area to the east of the Asir plate

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ANNEX 3 Results of physical and laboratory examinations of dogs collected from Mahayel, Asir Province.

Dog	Sex	Physical Examination				Laboratory Examination					
						Smears[3]		Sections[4]			
		Size[1]	Lesions[2]	Hpm*	Spm*	Liver	Spleen	Liver	Spleen	Culture[5]	ELISA[6]
D1	F	40x80	-	-	+	-	-	-	-	-	-
D2	F	30x70	-	-	-	-	-	-	-	-	-
D3	M	49x90	-	-	-	-	-	-	-	-	-
D4	M	40x60	E	-	-	-	-	-	-	-	-
D5	M	45x60	-	-	-	-	-	-	-	-	-
D6	F	35x55	-	-	-	-	-	-	-	-	-
D7	F	45x65	-	-	-	-	-	-	-	-	-
D8	M	45x70	-	-	-	-	-	-	-	-	-
D9	M	40x60	-	-	-	-	-	-	-	-	-
D10	M	60x86	E	-	-	-	-	-	-	-	+
D11	M	46x90	E	-	-	-	-	-	-	-	-
D12	M	47x60	-	-	-	-	-	-	-	-	+
D13	M	49x88	-	-	-	-	-	-	-	-	-
D14	M	31x54	-	-	-	-	-	-	-	-	-
D15	M	47x88	-	-	-	+	+	+	+	+	+
D16	M	47x88	-	-	-	-	-	-	-	-	-
D17	M	38x75	-	-	-	-	-	-	-	-	-
D18	F	46x75	-	-	-	-	-	-	-	-	-
D19	F	52x74	E	-	+	+	+	+	+	+	+
D20	F	45x75	-	-	-	+	+	+	+	+	+

Notes:

1. Size: height (cm) x length from nose to tail (cm)
2. Lesions: E-ear, S-skin
3. Impression smears: stained with Giemsa
4. Tissue sections: stained by the immunoperoxidase technique
5. Cultures: the positive cultures from dogs D15, D19 and D20 were highly contaminated and were not isoenzyme typed
6. ELISA: The ELISA technique was applied to blood spots

*Hpm: Heptomegaly, Spm: Splenomegaly

Dog D19 had thin legs and lesions in both ears

ANNEX 3 (continued) Results of physical and laboratory examinations of dogs collected from Mahayel, Asir Province.

		Physical Examination				Laboratory Examinations					
						Smears[3]		Sections[4]			
Dog	Sex	Size[1]	Lesions[2]	Hpm*	Spm*	Liver	Spleen	Liver	Spleen	Culture[5]	ELISA[6]
D21	M	40x60	-	-	-	-	-	-	-	-	-
D22	F	47x73	-	-	+	-	-	-	-	-	-
D23	F	34x70	-	-	-	-	-	-	-	-	-
D24	M	37x76	-	-	-	-	-	-	-	-	-
D25	M	50x90	-	-	-	-	-	-	-	-	-
D26	F	48x80	-	-	+	-	-	-	-	-	-
D27	F	55x85	-	-	-	-	-	-	-	-	-
D28	F	50x85	-	-	+	-	-	-	-	-	-
D29	F	43x66	-	-	-	-	-	-	-	-	-
F30	F	50x85	-	-	-	-	-	-	-	-	-
D31	F	60x85	-	-	-	-	-	-	-	-	-
D32	F	45x87	-	-	+	-	-	-	-	-	-
D33	F	40x70	-	-	-	-	-	-	-	-	-
D34	M	48x80	-	-	+	-	-	-	-	-	-
D35	M	45x75	-	-	-	-	-	-	-	-	-
D36	M	45x75	-	-	-	-	-	-	-	-	-
D37	M	40x70	-	-	-	-	-	-	-	-	-
D38	F	57x73	E	-	-	-	-	-	-	-	-
D39	F	50x82	-	-	-	-	-	-	-	-	-
D40	F	48x94	-	-	-	-	-	-	-	-	-

Notes:

1. Size: height (cm) x length from nose to tail (cm)
2. Lesions: E-ear, S-skin
3. Impression smears: stained with Giemsa
4. Tissue sections: stained by the immunoperoxidase technique
5. Cultures: the positive cultures from dogs D15, D19 and D20 were highly contaminated and were not isoenzyme typed
6. ELISA: The ELISA technique was applied to blood spots

*Hpm: Heptomegaly, Spm: Splenomegaly

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ANNEX 4 Results of physical and laboratory examinations of dogs collected from Al-Khoba, Gizan Province.

		Physical Examination				Laboratory Examinations					
						Smears[3]		Sections[4]			
Dog	Sex	Size[1]	Lesions[2]	Hpm*	Spm*	Liver	Spleen	Liver	Spleen	Culture[5]	ELISA[6]
D43	M	46x113	-	-	-	-	-	-	-	-	-
D44	M	48x116	-	-	-	-	-	-	-	-	-
D45	F	49x111	-	-	-	-	-	-	-	-	-
D46	M	51x113	-	-	-	-	-	-	-	-	-
D47	M	55x125	-	-	+	-	-	-	-	-	-
D48	F	47x116	-	-	-	-	-	-	-	-	-
D49	M	46x124	-	-	-	-	-	-	-	-	-
D50	M	42x106	-	+	+	-	-	-	-	-	+
D51	M	53x124	-	+	+	-	-	-	-	-	-
D52	M	53x126	-	-	-	-	-	-	-	-	-
D53	M	51x115	-	-	-	-	-	-	-	-	-
D54	F	51x116	-	-	-	-	-	-	-	-	+
D55	F	50x118	-	-	-	-	-	-	-	-	-
D56	M	41x107	-	-	-	-	-	-	-	-	-
D57	F	49x118	-	-	-	-	-	-	-	-	+
D58	M	54x123	-	+	+	-	-	-	-	-	-
D59	F	41x93	-	+	+	-	-	-	-	-	-
D60	F	55x119	-	-	-	-	-	-	-	-	-

Notes:

1. Size: height (cm) x length from nose to tail (cm)
2. Lesions: E-ear, S-skin
3. Impression smears: stained with Giemsa
4. Tissue sections: stained by the immunoperoxidase technique
5. Cultures: the positive cultures from dogs D15, D19 and D20 were highly contaminated and were not isoenzyme typed
6. ELISA: The ELISA technique was applied to blood spots

*Hpm: Heptomegaly, Spm: Splenomegaly

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ANNEX 4 (continued) Results of physical and laboratory examinations of dogs collected from Al-Khoba, Gizan Province.

		Physical Examination				Laboratory Examinations					
						Smears[3]		Sections[4]			
Dog	Sex	Size[1]	Lesions[2]	Hpm*	Spm*	Liver	Spleen	Liver	Spleen	Culture[5]	ELISA[6]
D61	M	55x123	-	-	-	-	-	-	-	-	+
D62	F	50x116	-	-	-	-	-	-	-	-	-
D63	M	53x122	-	-	-	-	-	-	-	-	+
D64	F	50x120	-	-	-	-	-	-	-	-	-
D65	F	44x110	-	-	-	-	-	-	-	-	-
D66	F	41x95	-	-	-	-	-	-	-	-	-
D67	F	50x110	-	-	-	-	-	-	-	-	-
D68	M	44x105	-	-	-	-	-	-	-	-	+
D69	F	52x105	-	-	-	-	-	-	-	-	-
D70	F	47x150	-	-	-	-	-	-	-	-	-
D71	F	43x87	-	-	-	-	-	-	-	-	-
D72	M	56x122	-	-	-	-	-	-	-	-	-
D73	M	54x118	-	-	-	-	+	+	+	+	+
D74	F	46x97	-	-	-	-	-	-	-	+	-
D75	M	49x103	-	-	+	-	-	-	-	+	-
D76	M	52x112	E	-	-	+	+	+	+	+	+
D77	F	53x117	-	-	-	-	-	-	-	-	-
D78	M	53x111	-	-	-	-	-	-	-	-	-
D79	F	50x107	-	-	+	-	-	-	-	-	+
D80	M	55x115	S	+	+	-	-	-	-	-	+

Notes:
1. Size: height (cm) x length from nose to tail (cm)
2. Lesions: E-ear, S-skin
3. Impression smears: stained with Giemsa
4. Tissue sections: stained by the immunoperoxidase technique
5. Cultures: the positive cultures from dogs D15, D19 and D20 were highly contaminated and were not isoenzyme typed
6. ELISA: The ELISA technique was applied to blood spots

*Hpm: Heptomegaly, Spm: Splenomegaly

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ANNEX 4 (continued) Results of physical and laboratory examinations of dogs collected from Al-Khoba, Gizan Province.

		Physical Examination				Laboratory Examinations					
						Smears[3]		Sections[4]			
Dog	Sex	Size[1]	Lesions[2]	Hpm*	Spm*	Liver	Spleen	Liver	Spleen	Culture[5]	ELISA[6]
D81	M	55x119	S	-	-	+	+	+	+	+	+
D82	M	56x123	-	-	-	-	-	-	-	-	-
D83	M	56x156	-	-	+	-	-	-	-	-	+
D84	F	51x108	-	-	-	-	-	-	-	-	-
D85	M	52x125	-	-	-	-	-	-	-	-	-
D86	M	51x115	-	-	+	-	-	-	-	-	-
D87	F	45x98	-	-	-	-	-	-	-	-	-
D88	M	54x120	-	-	-	-	-	-	-	-	-
D89	M	51x106	-	-	-	-	-	-	-	-	-
D90	F	55x117	-	-	-	-	-	-	-	-	-
D91	F	55x103	-	-	-	-	-	-	-	-	-

Notes:
1. Size: height (cm) x length from nose to tail (cm)
2. Lesions: E-ear, S-skin
3. Impression smears: stained with Giemsa
4. Tissue sections: stained by the immunoperoxidase technique
5. Cultures: the positive cultures from dogs D15, D19 and D20 were highly contaminated and were not isoenzyme typed
6. ELISA: The ELISA technique was applied to blood spots

*Hpm: Heptomegaly, Spm: Splenomegaly

Dog D81 had lesions on the thigh and lips

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ANNEX 5 Sandflies of the genera Phlebotomus and Sergentomyia captured using indoor light traps and sticky traps (December 1986 - December 1987).

Fixed station: Shuhat | Province: Asir
Location: Highland | Altitude: 2060m

Month	No. Traps	Phlebotomus species: arabicus M	F	sergenti M	F	orientalis M	F	bergeroti M	F	Genus Sergentomyia M	F	Total M	F
Dec	137	1	0	0	0	0	0	0	0	0	0	1	0
Jan	146	3	3	2	1	6	1	0	0	2	0	13	5
Feb	128	2	2	2	2	2	1	0	0	2	5	8	10
Mar	142	5	0	0	0	5	0	0	1	0	0	10	1
Apr	128	3	0	1	5	0	0	0	1	0	0	4	6
May	128	5	6	4	14	0	0	0	7	2	5	11	32
Jun	133	7	1	56	22	4	2	1	2	8	4	76	31
Jul	141	26	6	60	76	8	3	16	8	13	15	123	108
Aug	131	26	4	49	28	1	1	1	1	0	3	77	37
Sep	128	8	1	24	12	3	1	12	4	3	0	50	18
Oct	128	8	2	25	13	5	1	10	7	4	7	52	30
Nov	128	6	1	21	4	2	0	8	1	1	0	38	6
Dec	128	3	2	1	1	1	0	0	1	3	0	8	4
Total:		103	28	245	178	37	10	48	33	38	39	471	288

ANNEX 6 Sandflies of the genera Phlebotomus and Sergentomyia captured using outdoor light traps and sticky traps (December 1986 - December 1987).

Fixed station: Shuhat
Location: Highland

Province: Asir
Altitude: 2060m

Month	No. Traps	Phlebotomus species: arabicus		sergenti		orientalis		bergeroti		Genus Sergentomyia		Total	
		M	F	M	F	M	F	M	F	M	F	M	F
Dec	126	2	0	0	0	3	0	0	0	0	0	5	0
Jan	145	5	3	1	0	4	1	1	0	0	0	11	4
Feb	92	2	1	0	1	3	0	3	3	2	5	10	10
Mar	78	0	0	0	0	0	0	0	0	0	0	0	0
Apr	128	8	3	0	2	0	2	0	0	0	1	8	8
May	128	10	0	6	13	0	1	0	4	7	1	23	19
Jun	129	3	0	20	7	0	0	2	2	6	4	31	13
Jul	128	21	1	20	29	6	0	8	8	9	11	64	49
Aug	126	24	4	28	41	2	2	8	1	3	2	65	50
Sep	128	7	1	25	16	4	2	2	1	3	0	41	20
Oct	128	7	1	40	8	1	0	6	1	3	6	57	16
Nov	128	1	1	3	2	2	1	3	0	1	1	10	5
Dec	128	1	1	0	0	0	1	1	0	3	0	5	2
Total:		91	16	143	119	25	10	34	20	37	31	330	196

ANNEX 7 Sandflies of the genera Phlebotomus and Sergentomyia captured using indoor light traps (December 1986 - December 1987).

Fixed station: Shuhat Province: Asir
Location: Highland Altitude: 2060m

Month	No. Traps	Phlebotomus species: arabicus		sergenti		orientalis		bergeroti		Genus Sergentomyia		Total	
		M	F	M	F	M	F	M	F	M	F	M	F
Dec	6	0	0	0	0	0	0	0	0	0	0	0	0
Jan	7	1	0	0	0	0	0	0	0	2	0	3	0
Feb	8	0	2	0	2	0	0	0	0	0	0	0	4
Mar	8	0	0	0	0	5	0	0	1	0	0	5	1
Apr	8	0	0	0	4	0	0	0	1	0	0	0	5
May	8	2	3	0	14	0	0	0	7	1	2	3	26
Jun	10	6	1	35	16	4	2	0	2	6	2	51	23
Jul	8	23	6	56	72	0	3	5	3	4	5	88	89
Aug	8	26	4	41	27	1	1	1	1	0	1	69	34
Sep	7	6	1	7	11	3	1	5	3	0	0	21	16
Oct	8	5	2	11	11	5	1	6	4	1	1	28	19
Nov	8	5	1	2	3	2	0	2	1	1	0	12	5
Dec	6	1	2	0	1	0	0	0	1	0	0	1	4
Total:		75	22	152	161	20	8	19	24	15	11	281	226

ANNEX 8 Sandflies of the genera Phlebotomus and Sergentomyia captured using outdoor light traps (December 1986 - December 1987).

Fixed station: Shuhat — Province: Asir
Location: Highland — Altitude: 2060m

Month	No. Traps	Phlebotomus species: arabicus		sergenti		orientalis		bergeroti		Genus Sergentomyia		Total	
		M	F	M	F	M	F	M	F	M	F	M	F
Dec	6	2	0	0	0	0	0	0	0	0	0	2	0
Jan	6	0	0	0	0	3	0	0	0	0	0	3	0
Feb	8	2	0	0	0	0	0	0	3	0	0	2	3
Mar	8	0	0	0	0	0	0	0	0	0	0	0	0
Apr	8	8	2	0	2	0	0	0	0	1	0	9	4
May	8	5	0	2	13	0	1	0	4	5	1	12	19
Jun	8	1	0	3	4	0	0	0	2	1	0	5	6
Jul	8	19	1	18	27	3	0	5	6	0	0	45	34
Aug	7	19	2	21	27	2	2	0	0	3	1	45	32
Sep	7	7	0	17	15	4	2	0	0	0	0	28	17
Oct	8	2	0	4	3	1	0	2	1	0	0	9	4
Nov	10	1	1	3	0	1	1	0	0	1	1	6	3
Dec	6	0	1	0	0	1	1	0	0	0	0	1	2
Total:		66	7	68	91	15	7	7	16	11	3	167	124

ANNEX 9 Sandflies of the genera Phlebotomus and Sergentomyia captured using indoor sticky traps (December 1986 - December 1987).

Fixed station: Shuhat
Location: Highland

Province: Asir
Altitude: 2060m

Month	No. Traps	Phlebotomus species: arabicus		sergenti		orientalis		bergeroti		Genus Sergentomyia		Total	
		M	F	M	F	M	F	M	F	M	F	M	F
Dec	130	1	0	0	0	0	0	0	0	0	0	1	0
Jan	140	2	3	2	1	6	1	0	0	0	0	10	5
Feb	120	2	0	2	0	2	1	0	0	2	5	8	6
Mar	130	5	0	0	0	0	0	0	0	0	0	5	0
Apr	120	3	0	1	1	0	0	0	0	0	0	4	6
May	120	3	3	4	0	0	0	0	0	1	3	8	1
Jun	120	1	0	21	6	0	0	1	0	2	2	25	8
Jul	130	3	0	4	4	8	0	11	3	9	10	35	17
Aug	120	0	0	8	1	0	0	0	0	0	2	8	3
Sep	120	2	0	17	1	0	0	6	1	3	0	28	2
Oct	120	3	0	14	2	0	0	4	3	3	6	24	11
Nov	120	1	0	19	1	0	0	6	0	0	0	26	1
Dec	120	2	0	1	0	1	0	0	0	3	0	7	0
Total:		28	6	93	17	17	2	28	7	23	28	189	60

ANNEX 10 Sandflies of the genera Phlebotomus and Sergentomyia captured using outdoor sticky traps (December 1986 - December 1987).

Fixed station: Shuhat — Province: Asir
Location: Highland — Altitude:

Month	No. Traps	Phlebotomus species: arabicus		sergenti		orientalis		bergeroti		Genus Sergentomyia		Total	
		M	F	M	F	M	F	M	F	M	F	M	F
Dec	120	0	0	0	0	3	0	0	0	0	0	3	0
Jan	140	5	3	1	0	1	1	1	0	0	0	8	4
Feb	120	0	1	0	1	3	1	3	0	2	5	8	8
Mar	120	0	0	0	0	0	0	0	0	0	0	0	0
Apr	120	0	1	0	0	0	2	0	0	0	0	0	3
May	120	5	0	4	0	0	1	0	0	2	0	11	1
Jun	120	2	0	17	3	0	0	2	0	6	3	27	6
Jul	120	2	0	2	2	3	1	3	2	9	11	19	16
Aug	120	5	2	7	14	0	0	8	1	0	1	20	18
Sep	120	0	1	8	1	0	0	2	1	3	0	13	3
Oct	120	5	1	36	5	0	0	4	0	3	6	48	12
Nov	120	0	0	0	2	1	0	3	0	0	0	4	2
Dec	120	1	0	0	0	0	0	1	0	3	0	5	0
Total:		25	9	75	28	11	6	27	4	28	26	166	73

ANNEX 11 Sandflies of the genera Phlebotomus and Sergentomyia captured using indoor light traps and sticky traps (December 1986 - December 1987).

Fixed station: Bani-Thwa
Location: Lowland

Province: Asir
Altitude: 680m

Month	Phlebotomus species: alexandri		bergeroti		sergenti		Genus Sergentomyia		Total	
	M	F	M	F	M	F	M	F	M	F
Dec	4	2	39	32	0	0	9	22	52	56
Jan	0	0	31	78	5	8	21	28	57	114
Feb	2	2	16	9	7	12	32	45	57	68
Mar	0	2	7	27	4	9	9	38	20	76
Apr	0	0	14	62	4	6	7	39	25	107
May	1	1	25	63	17	16	50	84	93	164
Jun	1	3	51	61	13	13	49	81	114	158
Jul	0	5	92	119	8	1	31	75	131	200
Aug	3	4	59	35	8	1	20	17	90	57
Sep	4	3	75	75	14	12	237	181	330	271
Oct	0	1	45	28	7	3	68	90	120	122
Nov	3	0	45	20	8	3	71	88	127	111
Dec	2	0	11	9	1	0	7	6	21	15
Total:	20	23	510	618	96	84	611	794	1237	1519

ANNEX 12 Sandflies of the genera Phlebotomus and Sergentomyia captured using outdoor light traps and sticky traps (December 1986 - December 1987).

Fixed station: Bani-Thwa Province: Asir
Location: Lowland Altitude: 680m

Month	Phlebotomus species: alexandri		bergeroti		sergenti		Genus Sergentomyia		Total	
	M	F	M	F	M	F	M	F	M	F
Dec	0	0	40	18	3	2	16	11	59	31
Jan	0	1	16	4	6	4	11	16	33	25
Feb	2	2	7	11	1	1	26	54	36	68
Mar	1	3	14	34	2	8	21	57	38	102
Apr	0	0	10	64	1	2	8	20	19	86
May	3	1	26	119	3	15	26	87	58	222
Jun	3	4	49	90	9	7	32	83	93	184
Jul	1	6	82	146	8	7	18	43	109	202
Aug	0	4	63	125	6	3	10	17	79	149
Sep	1	2	100	146	9	4	302	199	412	351
Oct	2	4	22	16	5	0	29	63	58	83
Nov	3	0	22	17	2	2	48	66	75	85
Dec	3	0	11	4	0	0	12	16	26	20
Total:	19	27	462	794	55	55	559	732	1095	160

ANNEX 13 Sandflies of the genera Phlebotomus and Sergentomyia captured using indoor light traps (December 1986 - December 1987).

Fixed station: Bani-Thwa — Province: Asir
Location: Lowland — Altitude: 680m

Month	No. Traps	Phlebotomus species: alexandri M	alexandri F	bergeroti M	bergeroti F	sergenti M	sergenti F	Genus Sergentomyia M	Sergentomyia F	Total M	Total F
Dec	7	4	1	1	3	0	0	0	0	5	4
Jan	7	0	0	2	15	1	6	1	7	4	28
Feb	10	1	1	4	3	2	8	8	24	15	36
Mar	10	0	2	4	24	2	8	6	11	12	45
Apr	8	0	0	12	61	4	5	3	35	19	101
May	8	1	1	2	46	1	7	0	38	4	92
Jun	8	0	0	25	42	9	8	19	28	53	78
Jul	8	0	5	35	100	0	1	3	11	38	117
Aug	10	3	2	35	31	4	1	9	17	51	51
Sep	12	2	1	32	55	2	11	210	130	246	197
Oct	12	0	0	19	22	0	0	50	56	69	78
Nov	11	0	0	8	12	0	0	1	1	9	13
Dec	11	0	0	1	2	0	0	0	0	1	2
Total:		11	13	180	416	25	55	310	358	526	842

ANNEX 14 Sandflies of the genera Phlebotomus and Sergentomyia captured using outdoor light traps (December 1986 - December 1987).

Fixed station: Bani-Thwa
Location: Lowland

Province: Asir
Altitude: 680m

Month	No. Traps	Phlebotomus species: alexandri		bergeroti		sergenti		Genus Sergentomyia		Total	
		M	F	M	F	M	F	M	F	M	F
Dec	8	0	0	10	7	0	2	3	4	13	13
Jan	7	0	0	5	4	1	3	2	5	8	12
Feb	7	0	0	5	4	1	1	5	11	11	16
Mar	11	1	0	5	25	1	8	7	20	14	53
Apr	10	0	0	3	59	1	2	1	0	5	61
May	9	0	1	3	68	0	10	0	46	3	125
Jun	9	3	4	38	79	8	6	7	45	56	134
Jul	8	0	6	33	119	6	7	7	19	46	151
Aug	7	0	0	41	121	3	2	1	7	45	130
Sep	12	0	2	48	126	0	3	136	136	184	267
Oct	10	0	0	5	10	0	0	10	24	15	34
Nov	12	0	0	1	5	0	0	13	28	14	33
Dec	12	0	0	0	0	0	0	0	0	0	0
Total:		4	13	197	627	21	44	192	345	414	1029

ANNEX 15 Sandflies of the genera Phlebotomus and Sergentomyia captured using indoor sticky traps (December 1986 - December 1987).

Fixed station: Bani-Thwa | Province: Asir
Location: Lowland | Altitude: 680m

Month	No. Traps	Phlebotomus species: alexandri		bergeroti		sergenti		Genus Sergentomyia		Total	
		M	F	M	F	M	F	M	F	M	F
Dec	120	0	1	38	29	0	0	9	22	47	52
Jan	120	0	0	29	63	4	2	20	21	53	86
Feb	140	1	1	12	6	5	4	24	21	42	32
Mar	180	0	0	3	3	2	1	3	27	8	31
Apr	120	0	0	2	1	0	1	4	4	6	6
May	120	0	0	23	17	16	9	50	46	89	72
Jun	150	1	3	26	19	4	5	30	53	61	80
Jul	150	0	0	57	19	8	0	28	64	93	83
Aug	170	0	2	24	4	4	0	11	0	39	6
Sep	180	2	2	43	20	12	1	27	51	84	74
Oct	170	0	1	26	6	7	3	18	34	51	44
Nov	170	3	0	37	8	8	3	70	87	118	98
Dec	170	2	0	10	7	1	0	7	6	20	13
Total:		9	10	330	202	71	29	301	436	711	677

ANNEX 16 Sandflies of the genera Phlebotomus and Sergentomyia captured using outdoor sticky traps (December 1986 - December 1987).

Fixed station: Bani-Thwa   Province: Asir
Location: Lowland   Altitude: 680m

Month	No. Traps	Phlebotomus species: alexandri M	alexandri F	bergeroti M	bergeroti F	sergenti M	sergenti F	Genus Sergentomyia M	Sergentomyia F	Total M	Total F
Dec	120	0	0	30	11	3	0	13	7	46	18
Jan	140	0	1	11	0	5	1	9	11	25	13
Feb	150	2	2	2	7	0	0	21	43	25	52
Mar	190	0	3	9	9	1	0	13	37	23	49
Apr	130	0	0	7	5	0	0	7	20	14	25
May	130	3	0	23	51	3	5	26	41	55	97
Jun	160	0	0	11	11	1	1	25	38	37	50
Jul	140	1	0	49	27	2	0	11	24	63	51
Aug	180	0	4	22	4	3	1	9	10	34	19
Sep	180	1	0	52	20	9	1	166	63	228	84
Oct	170	2	4	17	6	5	0	19	39	43	49
Nov	160	3	0	21	12	2	2	35	38	61	52
Dec	160	3	0	11	4	0	0	12	16	26	20
Total:		15	14	265	167	34	11	366	387	680	579

ANNEX 17

TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (1988) 82, 416

Short Report

Phlebotomus sergenti, a vector of *Leishmania tropica* in Saudi Arabia.

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The presence of human infections with *Leishmania tropica* in the Kingdom of Saudi Arabia has recently been reported by PETERS *et al.* (1985). One strain (MHOM/SA/68/STIEHL), of undefined geographical origin within the Kingdom, obtained from a cutaneous leishmaniasis (CL) lesion in an expatriate and designated zymodeme LON22, showed a very similar isoenzyme profile to the international reference strain (MHOM/SU/60/OD). The second (MHOM/SA/83/GIZ33), which had a number of minor enzyme differences, from CL in a Saudi patient living on the Asir plateau, has been designated zymodeme LON63. They were the only representatives of this species among a large collection of Saudi Arabian strains, most of which were *L. major* acquired in the Eastern Province (PETERS *et al.*, 1985). Our current investigations have revealed that CL is widespread in villagers of the Asir plateau at an altitude of approximately 2000 m. Isozyme and monoclonal antibody characterization have revealed that the majority of isolates made so far are *L. tropica*, some similar or identical to zymodeme LON63, and others to zymodemes LON10 and LON71.

During the past year one of us (M.A.A.-Z.) has made regular collections of sandflies near the town of Abha, in which transmission of *L. tropica* is occurring at a high frequency, using miniature CDC light traps and castor oil traps. The identity of the sandflies collected in the area will be reported at a later date when all the material has been analysed. It can be confirmed, however, as earlier reported by BÜTTIKER *et al.* (1982), that *Phlebotomus sergenti* is present among the species of *Phlebotomus* in the endemic area. Of 140 *P. sergenti* dissected throughout the season, flagellates were found in the midgut of 2 specimens. These flies were collected by CDC light traps in houses in Shuhat and Mislat villages (altitude 2000 m) 20 and 30 km north of Abha City respectively. The flagellates were transferred to modified Tobie's medium (EVANS *et al.*, 1984) from which, after initial difficulty with bacterial contamination, pure cultures (ISER/SA/87/SSP286 and ISER/SA/87/SSP454) were obtained. Isoenzyme and monoclonal antibody typing confirmed that the organisms in each isolate were *L. tropica* corresponding to zymodeme LON10 (SSP286) and LON71 (SSP454), LON71 differing from LON10 in one enzyme (6PGDH) of the 12 examined. In addition to their isolation from *P. sergenti*, *L. tropica* organisms belonging to zymodemes LON71 (MHOM/SA/87/A126 and MHOM/SA/87/A127) and LON10 (MHOM/SA/87/B635) were isolated from human patients from the Asir plateau presenting with CL.

L. tropica has a wide distribution from the Mediterranean, through the Middle East and Central Asia, to the west of India and is probably an anthroponotic infection in most areas (ALJEBOORI & EVANS, 1980). *P. sergenti* is strongly suspected to be the vector in several countries but, because the precise identity of parasites found in this fly has apparently never before been confirmed by biochemical typing, the question has remained open. We are now able to confirm that, in the highlands of south-west Saudi Arabia, *P. sergenti* can be found infected with *L. tropica* which has biochemical characteristics in common with strains of this parasite originating in human CL cases in the same locality. Other sandfly species may also act as vectors. This and the question of possible animal reservoirs are being further investigated.

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References

Aljeboori, T. I. & Evans, D. A. (1980). *Leishmania* spp. in Iraq. Electrophoretic isoenzyme patterns. II. Cutaneous leishmaniasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **74**, 178-184.

Büttiker, W., Al-Ayed, I. H., Alwabil, A. H., Assalhy, H. S., Rashed, A. M. & Shareefi, O. M. (1982). A preliminary study on leishmaniasis in two areas of the Asir region. *Fauna of Saudi Arabia*, **4**, 509-519.

Evans, D. A., Lanham, S. M., Baldwin, C. I. & Peters, W. (1984). The isolation and isoenzyme characterization of *Leishmania braziliensis* subsp. from patients with cutaneous leishmaniasis acquired in Belize. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **78**, 35-42.

Peters, W., Elbihari, S., Ching Liu, Le Blancq, S. M., Evans, D. A., Killick-Kendrick, R., Smith, V. & Baldwin, C. I. (1985). *Leishmania* infecting man and wild animals in Saudi Arabia. 1. General survey. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **79**, 831-839.

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ANNEX 18

Leading Article

The Leishmaniases – a Public Health Problem in Saudi Arabia

Introduction

'Oriental sore' was reported by Manson as long ago as 1898 to occur in Arabia, although, at that time, the cause of the condition was unknown.[1] He pointed out that, as a rule, second attacks did not occur, a fact so well known in neighbouring Baghdad that a form of vaccination was practised there to avoid children developing ugly scars on their faces. Although the presence of parasites in skin lesions was first recognized by Cunningham in 1885, they were not named until several years later after similar organisms were seen in lesions from Indian patients suffering from the visceral type of leishmaniasis, 'kala-azar'. These were called *Piroplasma donovani* in 1903 by Laveran and Mesnil, a name soon to be changed by Ross to *Leishmania donovani*. For many years the name 'Leishman-Donovan' or LD bodies was given to the parasites seen in the tissues and this name is still found in many publications, even though it has long been pointed out that these stages should be called 'amastigotes', a term that will be used in this article. (See references 2 and 3 for interesting historical background data.)

The leishmaniases were either rare or rarely recognized in Saudi Arabia prior to about 1960. Phillips, in Egypt, in 1903 identified LD bodies in the spleen of two men, one who had lived for 15 years in Mecca and the other in Yemen.[4] An apparently autochthonous case of kala-azar (KA) was described in 1953 by Tarrizzo *et al.*[5] No data appeared on the distribution or prevalence of any autochthonous form of leishmaniasis in Arabia until ARAMCO health workers, in 1948, began to maintain records of diagnoses in hospital in-patients.[6] In their records for the 1960s are seven cases of cutaneous leishmaniasis (CL) which were considered to have been imported into the Eastern Province either from northern or central Saudi Arabia, or from other countries. However, from 1970 onwards, when their staff began to recognize the condition, an increasing number of cases was diagnosed to a total of 548 up to 1979. This increase, it should be noted, followed the successful drainage and malaria control scheme in Al-Hassa oasis, residual DDT house spraying being replaced after 1962 by anti-mosquito larviciding. In the meantime the area was undergoing a spectacular socioeconomic development including the construction of large numbers of new homes, farms and business premises.

The recognition of numerous cases of CL in the Eastern Province stimulated observations in other areas, including the agricultural region of Al Kharj and Riyadh where Morsy and his collaborators, Hawwary and Shoura, in a series of investigations, showed that intensive transmission was present. Subsequently a high prevalence rate of CL was reported from Bisha and Khamis Mushayt in the Asir region (see review in reference 7).

In March 1980 the first medical symposium on leishmaniasis to be held in the Kingdom was organized jointly by the Ministry of Health and the medical faculty of King Faisal University in Dammam.[8] At this meeting it became abundantly clear that CL was increasing rapidly in a number of areas of Saudi Arabia. Moreover, KA was by no means the rarity that it had been considered, a view that was reinforced at the 6th and 7th Saudi Medical Meetings in 1981 and 1982.[9,10] Whereas the majority of cases of KA originated in the southwest of the Kingdom, the existence of small foci of transmission in other localities could not be ruled out.

The Dammam symposium had two important consequences. First, the Ministry of Health became increasingly aware of the seriousness of the leishmaniases as a public health problem. Second, the National Leishmaniasis Research Programme (NLRP) was established in 1982 in King Faisal University under the sponsorship of the Saudi Arabian Council for Science and Technology (SANCST). This rapidly flowered into a multidisciplinary programme with participants from the Faculties of Medicine and Veterinary Science and collaboration with other experienced Saudi and overseas research workers.

What Causes Leishmaniasis

The term 'Leishmaniasis' itself is a misnomer since it embraces a variety of clinical syndromes that are, in turn, the outcome of infection with a wide variety of parasitic protozoa within the genus *Leishmania*. This genus, one of the flagellate Order known as the Kinetoplastida, is widely distributed throughout the tropics and subtropics of both the Old and New Worlds. Human infections, with few exceptions, are zoonotic, i.e., accidental infections by parasites which normally occur in wild animals that serve as reservoirs, the parasites being transmitted from one mammal to another by tiny sandflies of the genus *Phlebotomus*. A number of these, including *Phlebotomus papatasi*, a very common species in Saudi Arabia and neighbouring countries,[11] are extremely well adapted to life in desert or semi-desert terrain. In man several species of *Leishmania* can cause CL while others produce KA or attack mucous membranes or cartilage. It is better, therefore, to refer to "the leishmaniases" in discussing this very complex group of parasitic infections.

In the Old World the majority of CL is due to infection with one of two parasites, *L. major* which causes zoonotic cutaneous disease (ZCL) mainly in rural areas (although this is not always the case in Saudi Arabia) and *L. tropica*, the agent of urban CL. *L. major* is very widely distributed, causing CL in regions as far apart as Senegal in West Africa and Rajasthan in India. Urban CL due to *L. tropica*, prior to the extensive use of DDT as a residual spray in dwellings to kill the mosquito vectors of malaria, was very prevalent in many countries from the western Mediterranean and Mediterranean islands, across the near and middle East as far as Delhi in India. Unlike ZCL which is, by definition, a zoonosis, urban CL is essentially an anthroponosis in most countries; for this reason, its incidence has been considerably reduced by antimalarial house spraying. It is an entirely different story with ZCL since this involves many species of rodents as reservoirs and it has proved extremely difficult to interrupt the cycle of transmission to man by sandflies that normally feed on the wild rodents which inhabit complex burrows in the ground.

Until the last few years most of the literature on leishmaniasis has been obscured by the failure of investigators (physicians, especially dermatologists and immunologists, being the worst offenders) to recognize the existence of more than one species of *Leishmania*, even in a limited geographical area. The two species *L. tropica* and *L. major* of the Old World have long been recognized on biological grounds as being two distinct entities,[3] but this has not stopped some writers from confusing the literature

even up to the present day. The cause lies with the great similarity that exists between most leishmanial amastigotes when examined by normal light microscopy. It is now relatively easy to pinpoint the exact identity of *Leishmania* amastigotes in human or animal tissues or the 'promastigote' stages in the vectors or in culture medium by applying one or other of the biochemical techniques that are discussed below. At present, however, these still demand the expertise of a reference laboratory and are not at the stage where they can be applied by most medical staff at the bedside or out-patient clinic.

Leishmanial Syndromes and their Prevalence in Saudi Arabia

Cutaneous Leishmaniasis

Zoonotic CL is apparently the commonest type of tegumentary leishmaniasis in the Kingdom. However, in the absence of species identification, statistical data collected by the Leishmania Department of the Ministry of Health for the years 1978 to 1985 (Table 1) include all types of CL.[12] Most striking in this table is the great increase in the numbers reported year by year, partly due, perhaps, to the increasing awareness of CL but almost certainly also reflecting a true rise in prevalence. It should be noted, however, that a large proportion of diagnoses are based not on the demonstration of amastigotes in lesions but simply on clinical grounds. Nevertheless the total numbers reported (13 185 in 1985) are of serious proportions.

Table 1
Reported cases of cutaneous leishmaniasis in Saudi Arabia 1978 to 1985

Province	Number of cases reported							
	1978	1979	1980	1981	1982	1983	1984	1985
Al Baha	(Data reported with Western province)					395	1297	3062
Central (Riyadh)	92	130	235	445	618	4871	3127	2364
Eastern	701	579	1277	1049	1318	1646	1743	1663
Al Gasim	117	1239	1007	1225	2037	6047	3135	2013
Hail	84	158	298	215	154	3533	970	567
Al Medina	nil	244	506	249	543	895	877	1216
Asir	nil	33	69	88	299	810	1293	2144
Jaizan (Gizan)	(Reported with Asir and Southern Province)					8	nil	85
Najran						41	44	38
Western (Mecca)	49	86	129	246	414	33	52	18
Northern	nil	nil	nil	nil	nil	39	39	15
Totals	1043	2469	3521	3517	5383	18318	12577	13185

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In a recent survey, 52 out of 54 isolates of *Leishmania* from patients with CL who were infected in Saudi Arabia were proved by isoenzyme characterization to be *L. major*.[7] The majority of them were acquired in the Eastern Province. Of the other two, both of which were identified as *L. tropica*, one was acquired by a Saudi patient near Abha, and the other (in a foreign worker) in an unknown location. Although it is impossible to be certain on clinical grounds alone which of these two parasites has caused a particular lesion, *L. tropica* commonly produces single, slowly healing but relatively uncomplicated ulcers, often on the face, of a type observed fairly commonly in the southwest of Saudi Arabia. However, chronic lupoid leishmaniasis (leishmaniasis recidivans), which is a well-recognized syndrome produced by *L. tropica*,[13] does not appear to have been

reported from Saudi Arabia. In the Eastern province a number of infants have been observed with very severe facial lesions involving the mucous membranes of the nose and mouth such as those described by Al-Gindan and his associates from Hofuf.[14] The parasite isolated from their case was *L. major*. Certain species of *Leishmania* can bring about a specific immunological block which is manifested by the development of diffuse cutaneous lesions (DCL), resembling those of lepromatous leprosy, that are extremely refractory to therapy. No such cases have yet been found in Saudi Arabia. The normal response to CL caused by either *L. major* or *L. tropica* is a lifelong, cell-mediated immunity. While this prevents later infections from causing serious lesions, minor lesions may develop.[15] The leishmanin skin test is valuable in confirming the presence of immunity associated with current but chronic lesions or past infection.[16]

Visceral Leishmaniasis

Table 2
Reported cases of visceral leishmaniasis in Saudi Arabia 1980 to 1985

Province	Number of cases reported					
	1980	1981	1982	1983	1984	1985
Al Baha	-	-	-	8	4	nil
Central (Riyadh)	1	-	-	1	-	nil
Eastern	-	-	-	1	-	nil
Al Gasim	3	-	-	-	-	nil
Hail	-	-	-	-	-	nil
Al Medina	-	-	-	-	-	nil
Asir	-	-	1	10	41	98
Jaizan (Gizan)	4	2	2	15	43	90
Najran	-	-	-	-	-	nil
Western (Mecca)	2	3	2	3	3	nil
Northern	-	-	-	-	-	nil
Totals	10	5	5	38	91	188

(Unpublished data reproduced by permission of the Ministry of Health, Kingdom of Saudi Arabia)

Classical infantile kala-azar is being reported increasingly in the Kingdom, the majority of cases coming from the southwestern provinces (Table 2). Between 1980 and 1985 a total of 346 cases were notified to the Ministry of Health,[12] but this is likely to be a significant underestimate since the endemic area coincides in part with the areas in which malaria and schistosomiasis are also prevalent. Since the diagnosis is made often on clinical grounds, rather than on a more specific basis, such as seeing the amastigotes of *L. donovani* in biopsies of bone marrow or spleen,[16] the number reported is likely to include some false diagnoses. On the other hand, many acute cases are probably completely missed by inexperienced medical staff. At the same time, it is likely that other infections remain chronic or subclinical. The nature of the parasite has been confirmed to date by isoenzyme typing in the only two isolates available, both from infants in Gizan.[7] In the Mediterranean basin, several variants of the *L. donovani–L. infantum* complex have been shown to produce simple cutaneous lesions rather than KA. Although no such case has yet been identified in Saudi Arabia, this possibility should be kept in mind; nor have cases of post-kala-azar dermal leishmaniasis (PKDL) been reported in the Kingdom. Both PKDL and DCL should be considered in the differential diagnosis of patients with lesions suspected to be due to leprosy. While *M. leprae* is readily seen on tissue smears stained with Ziehl-Nielsen stain, material should be stained by the Giemsa technique[16] if the diagnosis is in doubt.

The immunological response to KA is primarily a humoral one, cell-mediated immunity (as reflected by the leishmanin test) only developing as a rule after the acute infection is cured.[17]

Species Diagnosis – Methods and Importance

Only recently has it been accepted that certain species of *Leishmania* (e.g. *L. aethiopica* in Ethiopia and Kenya and *L. braziliensis* in the new World) respond poorly to standard anti-leishmanial drugs and also that there can be considerable differences in the response of isolates of a particular parasite from different geographical areas. The problem has been how to distinguish the parasites, not only at the species level, but also at the level of such variations as response to drugs, antigens, and clinical outcome of infection. A variety of biochemical and immunological procedures are now becoming available with which to differentiate the *Leishmania*, most of which are extremely hard or impossible to identify on purely morphological grounds.[18] The methods currently in use are summarized in Table 3.

Table 3
Characters of value for the identification of Leishmania

A. INTRINSIC	
1 Genotypic	
DNA (nuclear, kinetoplast)	ultrastructure, size; G:C ratio; hybridization, endonuclease restriction analysis; DNA probes
2 Phenotypic	
2.1 Biochemical	
protein	primary structure (peptide map); secondary characters, isoenzymes, respiration, specific antigens
carbohydrate	primary structure; secondary characters (lectins, glycoprotein antigens)
lipids	primary structure
2.2 Morphological	gross and ultrastructure
B. EXTRINSIC	
1 Adaptation to environment	
1.1 Behaviour in culture	temperature optima; optimum media constituents
1.2 Behaviour in hosts	
vertebrate (man, wild or laboratory animals) invertebrate	parasitic or commensal; host specificity; site of election; pathology
1.3 Geographical distribution	
2 Response induced in hosts	
2.1 Antibodies	reaction to monoclonal antibodies
2.2 Cellular responses	Montenegro (leishmanin) test
2.3 Protective immunity	
2.4 Exoantigens	excretory factor serotyping
3 Response to chemical agents	
3.1 Baseline drug responses	*in vivo* and *in vitro*
3.2 Drug resistance patterns	

G:C = guanine:cytosine

Of the biochemical methods the characterization of isoenzymes has yielded the most valuable data on leishmanial taxonomy to date. Extensive surveys of Old World isolates

of *L. major*, *L. tropica* and the *L. donovani* complex have included material from Saudi Arabia and its neighbouring countries.[7,19,20,21] A standard form of coding has been

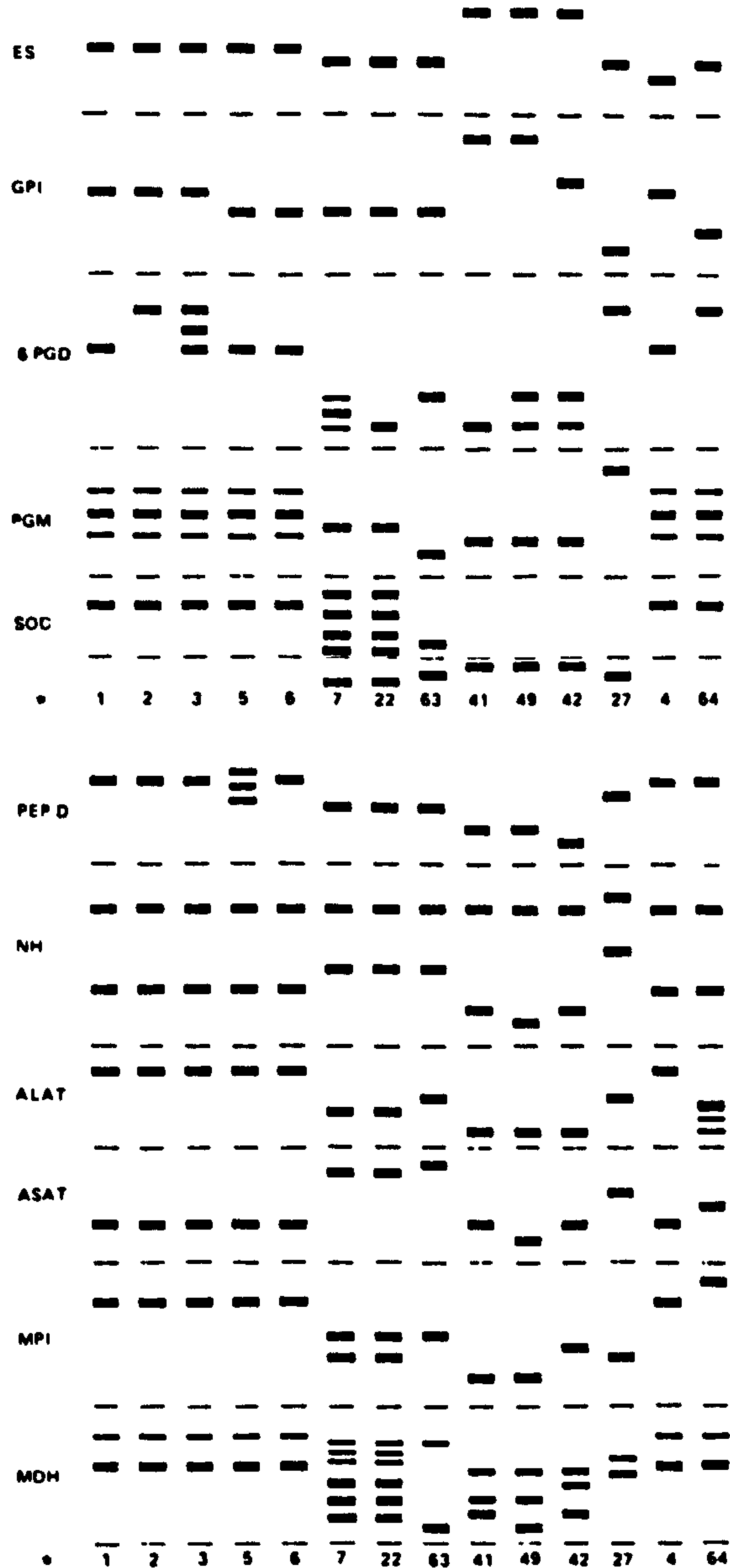

Figure 1. Diagrammatic representation of zymodemes of Leishmania *found in Saudi Arabia* compared with reference zymodemes as seen by starch gel electrophoresis. (Adapted from reference 7.) Key to enzymes: ES esterase; GPI glucosephosphate isomerase; 6-GPD 6-phosphogluconate dehydrogenase; PGM phosphoglucomutase; SOD superoxide dismutase; PEP D D-proline iminopeptidase; NH nucleoside hydrolase; ALAT alanine aminotransferase; ASAT aspartate aminotransferase; MPI mannosephosphate isomerase; MDH malate dehydrogenase. Key to zymodemes:* L. major *zymodemes LON-1, 2, 3, 4*, 5, 6;* L. tropica *zymodemes LON-7, 22*, 63*;* L. donovani *(sensu lato) zymodemes LON-41, 42*, 49;* L. aethiopica *zymodeme LON-27;* L. arabica *zymodeme LON-64*.*

adapted by the World Health Organization for isolates of *Leishmania* made from any source in order to avoid confusion when referring specimens for identification or in the literature; several WHO International Reference Centres for parasite identification have been established, including one in the senior author's laboratory.[22] From the patterns produced by a series of enzymes when separated from a parasite culture and submitted with a suitable method of electrophoresis (usually on thin starch gels or cellulose acetate papers), a profile is drawn up. This profile, referred to as a zymodeme, is compared to the profiles of standard marker isolates, of which a series has been agreed upon by WHO, in order to identify the parasites (Fig. 1). The method has proved invaluable but does, of course, necessitate the isolation of a strain either directly in culture medium or after primary isolation in a suitable experimental animal such as the golden hamster or mouse (BALB/c mice are invaluable for this purpose[24]).

Sophisticated techniques are being developed to permit the characterization of the DNA of *Leishmania*. These include the production of schizodemes from kinetoplast DNA that has been broken by appropriate endonucleases and, more recently, the development of specific DNA probes.[25] Initial studies with the latter have identified small numbers of amastigotes in cutaneous lesions by hybridization with a radioactive probe but, since this demands facilities that are not readily available in the field, non-radioactive DNA probes are now being studied, for example using biotinylated DNA.

A number of plant lectins that bind specifically to polysaccharides, glycoproteins and lipopolysaccharides on the surface of *Leishmania* have proved useful in identifying isolates but do not provide as fine a distinction as isoenzyme or kinetoplast DNA methods.[26] On the other hand, a variety of monoclonal antibodies raised against *Leishmania* are coming into use and are likely to contribute to the characterization of isolates, at least at the level of local reference laboratories.[27]

All these techniques have the advantage that they can be used to identify isolates from man or animals, and also from sandflies. Since the latter may transmit species of *Leishmania* and also other flagellates that are difficult to separate on microscopic grounds, it is obvious that biochemical techniques can be of great value in confirming the true vectors of leishmaniasis in a particular ecological situation.

For the clinician it is, of course, first of all important to attempt to find *Leishmania* in the relevant tissue in order to establish a firm diagnosis of leishmaniasis. Many cases have been submitted to prolonged treatment with anti-leishmanial drugs when they were suffering from other conditions and many have been misdiagnosed as other conditions and not received the correct antileishmanial therapy. While this may delay unnecessarily the cure of what is usually a self-healing disease (as with *L. major* infection), this is not true in an infant with kala-azar who may well die if not given specific chemotherapy.

In terms of prognosis it is important to differentiate between such parasites as *L. major* and *L. tropica* which, it must be emphasized, cannot be reliably differentiated on purely clinical grounds. Both tend to self-heal in time. However, inappropriate treatment of *L. tropica* in certain individuals can, in time, result in the development of leishmaniasis recidiva which may cause considerable disfigurement. In the New World infection with *L. braziliensis* may lead to a mutilating form of mucocutaneous leishmaniasis known as espundia, whereas parasites of the *L. mexicana* complex can result in incurable leishmaniasis diffusa (DCL).

Both the natural tendency towards self-cure and the different natural courses of infection with *L. major* and *L. tropica* make it essential to isolate and identify the parasites in order to evaluate correctly the outcome of clinical trials of new drugs or other forms of therapy. This has very rarely been done; one of the few exceptions being the current clinical trials of the NLRP in the Eastern Province.[28] This team has shown

that CL in the Al-Hassa area is due almost exclusively to *L. major* which is a variant (zymodeme LON-4) that is specific to Saudi Arabia and the neighbouring territory of Kuwait and Iraq.[19] In any future efforts to develop potent and specific vaccines against leishmanial infection in Saudi Arabia or elsewhere, it will be imperative to pinpoint the identity of the parasites present in the population to be protected, and also those being used in vaccine production.

The Determination of Animal Reservoirs and Vectors

Although by analogy with the epidemiology of CL in countries with a similar ecology to Saudi Arabia several investigators speculated on possible animal reservoirs, no accurate studies could be made until the parasites had been isolated and correctly identified. Thus the finding by Morsy and Shoura of amastigotes in a dog and two unidentified gerbils in the Al Kharj area in 1975[29] still left open the question of the parasites' identity and the possible role of the animals as reservoirs for human infection. So too did the observations by Büttiker and Lewis in 1979 as they only recorded lesions in rodents and dogs possibly due to *Leishmania* but made no actual parasitological study of them.[30] Büttiker[31] suggested that *Meriones crassus* and *M. libycus syrius* may serve as reservoirs in Al-Hassa and stated that *Psammomys obesus*, a well-known reservoir of *L. major* in other countries of North Africa and the Middle East[32] was absent there. The first positive identification of animal infections with *Leishmania* were those of Elbihari and his associates (members of the NLRP team) who, in 1984, succeeded in trapping 15 *Ps. obesus* in the Hofuf area of Al-Hassa and isolating *Leishmania* from six of them. None of 20 *M. crassus* nor the two *Mus musculus* examined proved positive.[33] Subsequently *L. major* zymodeme LON-4 was isolated from a total of 15 *Ps. obesus*, one *M. libycus* and one feral dog from the Al-Hassa area.[7] (While this was the first proof that the dog can serve as a natural host of this parasite, it is more likely that it, like man, is a victim rather than a natural reservoir host.) Moreover, a parasite new to science, *L. arabica*, was isolated from four *Ps. obesus* and another feral dog from the same area.[34] Unlike *L. major*, *L. arabica* has never, so far, been found in man.

Other members of the NLRP team carrying out intensive investigations into the phlebotomine fauna of the Al-Hassa area were able to confirm, for the first time in Saudi Arabia, that *Phlebotomus papatasi* is the vector there of *L. major* zymodeme LON-4[35] as Büttiker and Lewis[30] first speculated.

Treatment and Prevention of the Leishmaniases in Saudi Arabia

Treatment

The mainstay of therapy for all types of leishmaniasis remains the pentavalent antimonials, sodium stibogluconate (Pentostam) and meglumine antimoniate (Glucantime).[36] Critically conducted clinical trials by the NLRP team in the Eastern Province have shown that CL due to *L. major* zymodeme LON-4 is not fully responsive to the traditional standard dosage of sodium stibogluconate and alternative drugs and regimens are currently being investigated.

Many claims have been made for the efficacy of a wide variety of other drugs and alternative forms of therapy, such as cryosurgery in patients with CL, on the basis of completely uncontrolled trials in which no attempt has been made to identify the parasites nor to compare the test therapy with established regimens of known active agents. Such reports should be viewed very critically, especially since CL is almost always a self-limiting disease, albeit one that may require many months or some years for the complete resolution of lesions. There have been fewer claims of this nature for

the cure of KA which, in most cases, is not self-curing. In Saudi Arabia the infecting organism, one of the *L. donovani* complex, appears to be very sensitive to sodium stibogluconate which is the drug of choice.[16,22,36]

Prevention

Prevention of CL by vaccination with living parasites, a traditional practice in Iraq, has been carried out on an extensive scale in certain countries such as the USSR and is still being employed in Iran. It can produce lesions as serious as those of natural infection and has generally fallen out of favour.[22] In the absence of non-living vaccines for the prevention of the leishmaniases, control of these infections must depend on measures aimed at the vectors and or reservoir hosts, where these are known. In Saudi Arabia the NLRP know only the epidemiology of *L. major* in the Eastern Province, although it is likely that a similar situation exists in other areas with a similar ecology such as Al Kharj and Qasim. Nothing can be said at present about the nature of the parasites causing CL in the southwest of the Kingdom, the vectors in that region, nor possible animal reservoirs. It is obvious that the whole ecology of that region differs from that where *L.major* is transmitted by *P. papatasi*. Nor is there any information on the vectors or reservoirs of the parasites causing kala-azar or, indeed, the precise geographical distribution of this infection in Saudi Arabia. While it is believed that KA is a zoonosis and that transmission is limited to the southwest, this is by no means certain.

Experience in other countries with a similar ecology and epidemiology to that of the Hofuf area has shown that it is extremely difficult to interrupt permanently transmission of *L. major*. In Iran, for example, residual house spraying against malaria vectors only interrupted temporarily the transmission of CL.[37] The extent of the rodent reservoirs, as revealed in the Hofuf area, and the fact that the vector sandflies probably shelter in the rodent burrows for a large part of the day create a situation in which it is difficult to see how the transmission of CL can be interrupted without controlling the rodents themselves. Observations by the NLRP team and other workers have revealed that the principal animal reservoir, *Ps. obesus*, depends heavily for its nutrition on certain species of *Chenopodium* that grow profusely in sandy areas around the villages and farms. One way to eliminate rodents from the neighbourhood of habitations would be to remove their main food source since trapping of this animal is extremely difficult and unrewarding as a method of control. This method is currently being investigated.

Conclusions

The leishmaniases are clearly a significant and increasing public health problem in the Kingdom of Saudi Arabia. While many facets of the epidemiology and clinical aspects of CL caused by *L. major* in the Eastern Province have been revealed in the past few years by the efforts of the NLRP team and other investigators, information on the parasites present in other parts of the country and the epidemiological factors governing their transmission is very sparse. An intensive research programme paralleling that carried out by the NLRP is called for to investigate the situation, especially in the southwestern provinces of Asir, Al Baha and Gizan. Such a programme will call for adequate funding, a critical number of full-time key staff and the collaboration of one or more established laboratories in the study area. The full cooperation will be required of suitable academic centres as well as designated, trained staff of the Ministry of Health and other government bodies where appropriate (e.g. the clinical staff of military hospitals). If adequate personnel are not available within the Kingdom it may be necessary to seek assistance from outside experts or from WHO. It must be emphasized that the launching of such a programme will require a major and ongoing commitment

in terms of funding and personnel, as well as careful planning by experienced advisers. Any lessons learned will undoubtedly be of value to neighbouring countries with similar epidemiological patterns and hence provide an example of rational disease control.

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Saudi Medical Journal 1987; 8(4): 333–343

References

[1]Manson P. *Tropical diseases. A manual of the diseases of warm climates.* London, Paris, New York, Melbourne: Cassell & Co, 1898.
[2]Kean BH, Mott KE, Russell AJ, eds. *Tropical medicine and parasitology. Classic investigations*, Vol. 1. Ithaca, London: Cornell University Press, 1978.
[3]Lainson R, Shaw JJ. Evolution, systematics and distribution. In: Peters W, Killick-Kendrick R, eds. *The leishmaniases in biology and medicine*, Vol. 1. London: Academic Press, 1986: 1–120.
[4]Phillips L. Note on the occurrence of the Leishman-Donovan parasite in Arabia and Egypt. *J Trop Med Hyg* 1904; 7: 236–237.
[5]Tarrizzo ML, Bracken HA, Strait DJ. A case of visceral leishmaniasis in Saudi Arabia. *Am J Trop Med Hyg* 1953; 2: 846–849.
[6]Al Dafas AA, Mohammed CK. The epidemiology of cutaneous leishmaniasis in ARAMCO health care population. In: *Medical symposium on leishmaniasis. March 22–24, 1980, Dammam.* Riyadh: Ministry of Health, 1985: 147–160.
[7]Peters W, Elbihari S, Ching Liu, *et al. Leishmania* infecting man and wild animals in Saudi Arabia 1. General survey. *Trans R Soc Trop Med Hyg* 1985; 79: 831–839.
[8]Anonymous. *Medical symposium on leishmaniasis. March 22–24 1980, Dammam.* Riyadh: Ministry of Health, 1985.
[9]El-Bihairy F, Jan M, Kamel A, Omer A. Visceral leishmaniasis in children; report of 6 cases in Jeddah. In: *Abstracts, 6th Saudi medical meeting, 15–18 March 1981, Jeddah.* Jeddah: King Abdulaziz University, 1981: 152.
[10]El-Bihairy F, Jan MY, Dhoparee I, Omer A. Haematologic and serologic aspects of visceral leishmaniasis in children in Saudi Arabia. In: *Proc 7th Saudi medical meeting, 3–6 May 1982, Dammam.* Dammam: King Faisal University, 1982: 228–245.
[11]Lewis DJ, Büttiker W. Insects of Saudi Arabia. The taxonomy and distribution of Saudi Arabian phlebotomine sandflies (Diptera: Psychodidae). *Fauna of Saudi Arabia* 1982; 4: 353–397.
[12]Leishmania Department. *Annual report on Leishmania control programme 1985.* Unpublished data, Riyadh: Ministry of Health, 1985.
[13]Griffiths WAD. Old World cutaneous leishmaniasis. In: Peters W, Killick-Kendrick R, eds. *The leishmaniases in biology and medicine, Vol. 2.* London: Academic Press, 1986: 617–636.

[14]Al-Gindan Y, Omer AHS, Al-Humaidan Y, Peters W, Evans DA. A case of mucocutaneous leishmaniasis in Saudi Arabia caused by *Leishmania major* and its response to treatment. *Clin Exp Dermatol*. 1983; **8**: 185–188.

[15]Killick-Kendrick R, Bryceson ADM, Peters W, Evans DA, Leaney AJ, Rioux A-J. Zoonotic cutaneous leishmaniasis in Saudi Arabia: lesions healing naturally in man followed by a second infection with the same zymodeme of *Leishmania major*. *Trans R Soc Trop Med Hyg* 1985; **79**: 363–365.

[16]Peters W. Diagnostic procedures in leishmaniasis. In: *Medical symposium on leishmaniasis. March 22–24 1980, Dammam*. Riyadh: Ministry of Health, 1985: 30–56.

[17]Rees PH, Kager PA. Visceral and post-kala-azar dermal leishmaniasis. In: Peters W, Killick-Kendrick R, eds. *The leishmaniases in biology and medicine, Vol. 2*. London: Academic Press, 1986: 583–616.

[18]Chance ML, Walton BC, eds. *Biochemical characterization of Leishmania*, Geneva: UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, 1982.

[19]Le Blancq SM, Schnur LF, Peters W. *Leishmania* in the Old World: 1. The geographical and hostal distribution of *L. major* zymodemes. *Trans R Soc Trop Med Hyg* 1986; **80**: 99–112.

[20]Le Blancq SM, Peters W. *Leishmania* in the Old World: 2. Heterogeneity among *L. tropica* zymodemes. *Trans R Soc Trop Med Hyg* 1986; **80**: 113–119.

[21]Le Blancq SM, Peters W. *Leishmania* in the Old World: 4. The distribution of *L. donovani* sensu lato zymodemes. *Trans R Soc Trop med Hyg* 1986; **80**: 367–377.

[22]World Health Organization. *The leishmaniases. Techn Rep Ser* No. 701, Geneva: WHO, 1984.

[23]Chance ML. The biochemical and immunological taxonomy of *Leishmania*. In: Chang KD, Bray RS, eds. *Leishmaniasis*. New York: Elsevier Science Publishers, 1986: 93–110.

[24]Schnur LF, Jacobson RL. Parasitological techniques. In: Peters W, Killick-Kendrick R, eds. *The leishmaniases in biology and medicine*, Vol. 1. London: Academic Press: 1986: 499–542.

[25]Barker DC, Butcher J, Gibson LJ, Williams RH. *Characterization of Leishmania sp. by DNA hybridization probes. A laboratory manual*, Geneva: UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, 1986.

[26]Schottelius J. Lectin binding strain-specific carbohydrates on the cell surfaces of *Leishmania* strains from the Old World. *Z Parasitenk*. 1982; **66**: 237–247.

[27]McMahon Pratt D, David JR. Applications of monoclonal antibodies specific for *Leishmania* species. In: Chance ML, Walton BC, eds. *Biochemical characterization of Leishmania*. Geneva: UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, 1982: 247–257.

[28]Kubba R, Al-Gindan Y, El-Hassan AM, Omer AHS. Expanding clinical spectrum of cutaneous leishmaniasis in the Eastern Province. In: *Abstracts, 8th Saudi Medical Conference 30 Oct.–3 Nov. 1983, Riyadh*, Riyadh: King Saud University, p. 214.

[29]Morsy TA, Shoura MI. Natural *Leishmania* infection sought in animals in Al Kharj, Saudi Arabia. *J Egypt Publ Hlth Ass* 1975; **5**: 328–331.

[30]Büttiker W, Lewis DJ. Ecological studies at Hofuf, Eastern Saudi Arabia, in relation to dermal leishmaniasis. *Tropenmed Parasitol* 1979; **30**: 220–229.

[31]Büttiker W. Phlebotomine sandflies in Saudi Arabia. In: *Medical symposium on leishmaniasis. 22–24 March 1980, Dammam*. Riyadh: Ministry of Health, 1985: 178–201.

[32]Ashford RW, Bettini S. Ecology and epidemiology: Old World. In: Peters W, Killick-Kendrick R. eds. *The leishmaniases in biology and medicine*, Vol. 1. London: Academic Press, 1986: 365–424.

[33]Elbihari S, Kawasmeh ZA, Al Maiem AH. Possible reservoir host(s) of zoonotic cutaneous leishmaniasis in Al-Hassa oasis, Saudi Arabia. *Ann Trop Med Parasitol* 1985; **78**: 543–545.

[34]Peters W, Elbihari S, Evans DA. *Leishmania* infecting man and wild animals in Saudi Arabia 2. *Leishmania arabica* n.sp. *Trans R Soc Trop Med Hyg* 1986; **80**: 497–502.

[35]Killick-Kendrick R, Leaney AJ, Peters W, Rioux A-J, Bray RS. Zoonotic cutaneous leishmaniasis in Saudi Arabia: the incrimination of *Phlebotomus papatasi* as the vector in the Al-Hassa oasis. *Trans R Soc Trop Med Hyg* 1985; **79**: 252–255.

[36]Bryceson A. Therapy in man. In: Peters W, Killick-Kendrick R, eds. *The leishmaniases in biology and medicine*, Vol. 2. London: Academic Press, 1986: 847–908.

[37]Seyedi-Rashti MA, Nadim A. Re-establishment of cutaneous leishmaniasis after cessation of anti-malaria spraying. *Trop Geog Med* 1975; **27**: 79–82.

Annex 19

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Short Report

Visceral leishmaniasis in man and dogs in south-west Saudi Arabia.

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Notifications of human visceral leishmaniasis (kala-azar, VL) in the Kingdom of Saudi Arabia have increased dramatically from a total of 10 in 1980 to 247 in 1986 (PETERS & AL-ZAHRANI, 1987), although whether this is due to a true increase in the incidence of VL or an increased awareness of the diagnosis is uncertain at present. The disease in man occurs mainly in infants and young children living in or coming from the south-western provinces of the country. The nature of the causative organism was unknown before PETERS *et al.* (1985) reported the characterization by isoenzyme electrophoresis of a parasite referable to *Leishmania donovani sensu lato* which was labelled zymodeme LON-42. The parasite was isolated from one infant in Gizan and another in Jeddah who had originated from the Gizan area. No animal reservoirs of VL were known in Saudi Arabia.

Recently one of us (M.A.-Z.) has been able to examine a sample of the feral dog population in and around 2 active foci of human VL, both in the lower parts of the Asir range in south-west Saudi Arabia. The first was Beni Thwa, situated about 480 m above sea level in the Mahayel District of Asir Province. The second was the Al-Khoba area of Gizan Province, 175 m above sea level. Feral dogs, which are very abundant in these areas, living in close proximity to man and wandering around farm buildings and houses are considered to be a great nuisance by the local population. Official permission was obtained to capture some of the animals which, with the active collaboration of the local population and skilled marksmen were shot with darts containing Scoline® (suxamethonium), a muscle relaxant. Over 2 periods, each of 5 d, 89 feral dogs were captured, 40 from Beni Thwa and 49 from Al-Khoba.

The unconscious animals were taken to the field laboratory where they were killed, then examined and dissected within 30 min of death. Material was aspirated from the livers and spleens from which impression smears were made for direct microscopy after fixation and staining. Amastigotes were found in preparations from 6 of the 89 dogs and cultures were obtained from 3 of them. Isoenzyme characterisation using 12 enzymes (as described by LE BLANCQ & PETERS, 1985) showed that all 3 isolates were identical to each other and to the WHO recommended reference strain for *L. infantum* (MHOM/TN/80/IPT1), zymodeme LON-49 (=MON1).

While the study of the dogs was in progress, isolates were also obtained from bone marrow biopsies of infants with suspected VL in the same geographical areas. The isoenzyme characterization of 9 of these isolates showed that all corresponded to zymodeme LON-42 of *L. donovani sensu lato*. This zymodeme differs significantly from *L. infantum*, the MDH, ASAT, MPI, PEPD and NH zymograms being readily distinguished *L. infantum* zymodeme LON-49 was not found in isolates from any of these cases nor, indeed, from any human VL case in Saudi Arabia.

The discovery of different organisms causing human and canine VL in the identical geographical areas in south-west Saudi Arabia raises several important questions and stresses once more the importance of characterizing the organisms when the epidemiology of leishmaniasis is being investigated. In the Mediterranean littoral zone it has been clearly shown that *L. infantum* zymodeme LON-49 is responsible for both human and canine VL and that canids are the principal reservoir hosts of human VL (ASHFORD & BETTINI, 1987). A different situation is now seen to exist in south-west Saudi Arabia. Before carrying out the isoenzyme typing we had assumed, because we found dogs to be infected, that they were the reservoirs of human VL, but this assumption was immediately dispelled when organisms from the 2 hosts were shown to be quite distinct. RIOUX *et al.* (1986) have also reported the identification of '*L. donovani* zymodeme MON-31' in 2 infants with VL from lowland villages of the neighbouring Yemeni Arab Republic (MON-31 is synonymous with LON-42). If true *L. infantum* does not infect humans in this part of the Arabian peninsula it may be because its sandfly vector (as yet unidentified there) rarely bites man. The search is continuing for the vectors of both the human and canine pathogen and for the animal reservoirs of *L. donovani* zymodeme LON-42.

Full acknowledgements to the many people involved will be made in the detailed report which is in preparation. M.A.-Z., who is on study leave from the Ministry of Health, is grateful to Dr Jalal Ashi, Deputy Minister of Health and Dr M. Al-Jiffry, Director of the Leishmaniasis Division, for their help and support with this study.

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References

Ashford, R. W. & Bettini, S. (1987). Ecology and epidemiology: Old World. In: *The Leishmaniases in Biology and Medicine*, (Peters, W. & Killick-Kendrick, R., editors). London: Academic Press, Vol. 1, pp. 365–424.

Le Blancq, S. M. & Peters, W. (1985). *Leishmania* in the Old World: 4. The distribution of *L. donovani sensu lato* zymodemes. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 80, 367–377.

Peters, W. & Al-Zahrani, M. A. (1987). The leishmaniases—a public health problem in Saudi Arabia. *Saudi Medical Journal*, 8, 333–343.

Peters, W., Elbihari, S., Ching Liu, Le Blancq, S. M., Evans, D. A., Killick-Kendrick, R., Smith, V. & Baldwin, C. I. (1985). *Leishmania* infecting man and wild animals in Saudi Arabia. 1. General survey. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 79, 831–839.

Rioux, J.-A., Daoud, W., Pratlong, F., El Kubati, Y., Moreno, G., Rageh, H. A., Brun, R., Mouharem, A., Martinez Ortega, E. & Belmonte, A. (1986). Les complexes *Leishmania donovani* s.st., *Leishmania tropica*

et *Leishmania major* en République Arabe de Yémen. Considérations taxonomique et épidémiologiques. In: Leishmania. *Taxonomie–Phylogenèse*. (Rioux, J.-A. editor). Montpellier: Institut Méditerranéen d'Etudes Epidémiologiques et Ecologiques, pp. 357–363.

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