INVESTIGATION ON CUTANEOUS LEISHMANIASIS IN MEN AND DOGS IN BIKANER, RAJASTHAN

राजस्थान के बीकानेर शहर में मनुष्यों एवं कुत्तों में क्यूटेनियस लेश्मानिएसिस का अन्वेषण

A THESIS
PRESENTED TO
The Faculty of Veterinary and Animal Science
RAJASTHAN AGRICULTURAL UNIVERSITY
BIKANER

In partial fulfilment
of the requirements for the Degree of
Master of Veterinary Science
(Veterinary Public Health)

By
Surendra Kumar Chhangani
B.V.Sc. & A.H.

1993

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RAJASTHAN AGRICULTURAL UNIVERSITY, BIKANER

CERTIFICATE - I

This is to certify that Mr. Surendra Kumar Chhangani has successfully completed the COMPREHENSIVE EXAMINATION held on 25th September, 1992 as required under the regulations for Master of Veterinary Science.

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CERTIFICATE - II

Date : 06/4/93

This is to certify that this thesis entitled "Investigation on Cutaneous Leishmaniasis in men and dogs in Bikaner, Rajasthan" submitted for the degree of Master of Veterinary Science, in the subject of Veterinary Public Health of the Rajasthan Agricultural University, Bikaner is a bonafide research work carried out by Mr. Surendra Kumar Chhangani B.V.Sc. & A.H. under my supervision and that no part of this thesis has been submitted for any other degree. The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE - III

Date : 22.9.93.

This is to certify that the thesis entitled "Investigation on cutaneous leishmaniasis in men and dogs in Bikaner, Rajasthan." submitted by Mr. Surendra Kumar Chhangani, B.V.Sc. & A.H. to the Rajasthan Agricultural University, Bikaner in partial fulfilment of the requirements for the degree of Master of Veterinary Science in the subject of Veterinary Public Health has been approved by the Student's Advisory committee after an oral examination on the same in collaboration with an external examination.

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(M.S. Vashishtha)
Member, Advisory Committee

DEDICATED TO

MY AFFECTIONATE YOUNGER BROTHER

DR. MANOJ.

TO MY ESTEEMED ADVISOR

Words can hardly express my sense of indebtness to my esteemed advisor, my "GURU" Dr. S.K. Purohit, B.V.Sc. & A.H., M.V.Sc., Ph.D., Assistant Professor, Veterinary Public Health and Hygiene, Department of Medicine, for his precious supervision, scholastic guidance, invaluable inspiration so often sought and so generously given at the cost of his personal convenience.

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Surendra Kumar Chhangani

Investigation on cutaneous leishmaniasis in men and dogs in Bikaner, Rajasthan.

M.V.Sc. Thesis
Veterinary Public Health,
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Bikaner.

Submitted by : Surendra Kumar Chhangani

ABSTRACT

From the public health point of view cutaneous leishmaniasis is one of the most important zoonotic diseases of this area occurring mainly in dogs and men. It is caused by **Leishmania tropica** and transmitted through the bite of sandfly i.e. **Phlebotomus** species.

Presently cutaneous leishmaniasis infection is recorded for the first time in the pet dogs. In the present study a survey of 542 street and 98 pet dogs were made around the Bikaner city. Where 36 cases of cutaneous leishmaniasis in human being were reported. Out of 542 street dogs only 11 cases and out of 98 pet dogs only 6 cases are reported as cutaneous leishmaniasis. It seems that this disease is restricted only around the area of human patients of oriental sore. Disease was independent to age and sex. In maximum cases the lesions were observed on the extremities. Both intra and extracellular forms of **L.tropica** were present as revealed by the contact smear prepared from cutaneous lesions. The host cells were mononuclear leucocytes which were in certain cases found literally packed with parasite.

In the case of human being, 36 out of 74 persons are positive for oriental sore. Human population below 20 years age group showed high incidence. This disease was independent to sex. The duration of lesions was 1 - 2 months in maximum cases. Lesions were mainly confined over exposed parts of the body.

It is observed that cutaneous leishmaniasis infection is a localised skin infection as not a single case of **Leishmania tropica** body was observed in general circulation. In comparison to NNN media, Dephasic media was found to be superior for the cultivation of **L.tropica** where the parasite multiplied well at 48 hours in B.O.D. incubation at 22°C.

Five to eight injections of 2% aqueous solution of berbarian sulphate at weekly interval (1 ml. intradermally in each lesion) are found to be effective for proper healing of cutaneous lesions in men and dogs.

राजस्थान के बीकानेर शहर में मनुष्यों एवं कुत्तों में क्यूटेनियस लेश्मानिएसिस का अन्वेषण

स्नातकोत्तर शोध प्रबन्ध

पशु चिकित्सीय जन-स्वास्थ्य, औषध विभाग,

पशु चिकित्सा एवं पशु विज्ञान महाविद्यालय,

राजस्थान कृषि विश्वविद्यालय, बीकानेर.

प्रस्तुतकर्त्ता:- सुरेन्द्र कुमार छंगाणी

सं क्षे प

जन-स्वास्थ्य की दृष्टि से क्यूटेनियस लेश्मानिएसिस बीकानेर क्षेत्र का अति महत्वपूर्ण जूनोटिक रोग है, जो मुख्यतः मनुष्यों व कुत्तों में पाया जाता है । यह रोग लेश्मानिया ट्रोपिका के कारण होता है । यह सेंडफ्लाई यानि फ्लेबोटोमस जाति की मक्खी के काटने से फैलता है और इस अध्ययन के दौरान यह मक्खी लेश्मानिएसिस से ग्रसित रोगियों के घरों में पाई गयी। इस समय क्यूटेनियस लेश्मानिएसिस का प्रभाव पहली बार इस क्षेत्र के पालतू कुत्तों की विभिन्न नस्लों में भी देखा गया ।

प्रस्तुत अध्ययन में बीकानेर शहर के जिन क्षेत्रों में 36 मनुष्यों में क्यूटेनियस लेश्मानिएसिस का रोग देखा गया, उनके आसपास के मोहल्लों के 542 आवारा कुत्तों व 98 पालतू कुत्तों का भी सर्वेक्षण किया गया । उन 542 आवारा कुत्तों में से केवल 11 तथा 98 पालतू कुत्तों में से केवल 6 कुत्ते क्यूटेनियस लेश्मानिएसिस से ग्रसित पाये गये। इससे यह प्रतीत होता है कि यह रोग केवल उस क्षेत्र के आसपास ही सीमित रहता है, जिस क्षेत्र में ओरियन्टल सोर से मानव ग्रसित होता है। यह रोग सभी आयु के कुत्तों में

पाया गया। कुत्तों के लिंग पर रोग निर्भर नहीं था। अधिकांश मामलों में, कुत्तों के शरीर के बाल रहित भागों पर घाव पाये गये। क्यूटेनियस घावों से निर्मित स्पर्श स्मेयर से पता चला कि इनमें लेश्मानिया ट्रोपिका के दोनों अन्तः एवं बाह्य कोशिकीय, रूप विद्यमान थे। वे मुख्यतः रक्त के मोनोन्यूकलियर कोशिका में पाये गये।

इस अध्ययन के दौरान 74 संदिग्ध मानव रोगियों में से 36 रोगी 'ओरियन्टल सोर' से ग्रसित पाये गये। यह रोग 20 वर्ष से कम आयु वर्ग वाले लोगों में अत्यधिक देखा गया। मनुष्यों में भी यह रोग किसी लिंग पर निर्भर नहीं करता। अधिकांश मामलों में घाव की अवधि एक से दो महीने तक की थी। मनुष्यों में इस रोग के घाव शरीर के खुले भागों पर ही पाये गये।

यह देखा गया कि क्यूटेनियस लेश्मानिएसिस रोग मनुष्यों व कुत्तों के शरीर का एक स्थानिक चर्म रोग है, क्योंकि परीक्षण के दौरान लेश्मानिया ट्रोपिका बोडी का एक भी मामला सामान्य रक्त परिसंचरण में नहीं पाया गया। एन॰ एन॰ एन॰ मीडिया की तुलना में डाईफेजिक मीडिया को लेश्मानिया ट्रोपिका के कल्टीवेशन के लिये बेहतर पाया गया, क्योंकि इस मीडिया में परजीवी ने बी॰ ओ॰ डी॰ इन्कूबेटर में 22 डिग्री सेन्टीग्रेड तापमान पर 48 घण्टे में अच्छी वृद्धि की।

मनुष्यों एवं कुत्तों के क्यूटेनियस घावों को पूरी तरह भरने के लिये 2 प्रतिशत बरबेरियन सल्फेट के घोल के 5 से 8 इन्जेक्शन प्रभावकारी होते हैं। प्रत्येक घाव में एक मि॰ली॰ इन्टराडर्मली, 2 प्रतिशत बरबेरियन सल्फेट के घोल का इन्जेक्शन एक सप्ताह के अन्तराल पर देना उपयुक्त रहता है।

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INTRODUCTION

1. INTRODUCTION

Out of the various diseases occuring among men and animals, more than 300 diseases have been so far listed as intercommunicable between men and animals and out of these about 100 disease are of major Public Health importance. L. tropica, infection is one of these metazoonotic infection.

The leishmania infection is caused by protozoa. This is responsible for three types of distinctive diseases on account of skin manifestations in each namely : -

(i) Cutaneous leishmaniasis (Oriental sore).

(ii) Visceral leishmaniasis (Kala-azar).

(iii) Muco-cutaneous leishmaniasis.

Cutaneous leishmaniasis is known by various names as Oriental sore, Tropical sore, Bay sore, Datemark, Delhi boil etc. and is caused by Leishmania tropica.

The cutaneous leishmaniasis has been further classified into two types (Chandler and Read, 1961)

(i) "Dry type"

(ii) "Moist type"

The "Dry type" is a non or late ulcerating, chronic disease caused by L. tropica minor with incubation period of several months. Metastatic lesions and involvement of mucous membrane is uncommon. It is urban in distribution and only 10

per cent cases show involvement of lymphatic glands. This type of leishmaniasis is transmitted from men to men through sand flies. The sand flies commonly involved in its transmission are P. papatasii, P. sergenti, P. perfilieri in Italy and P. lengicuspis in Algeria (Chandler and Read, 1961).

The "Wet type" of cutaneous leishmaniasis is caused by L. tropica major. It has short incubation period of about 1-6 weeks. It is confined in the rural areas and ulcerates rapidly. Involvement of lymphatic glands is more common. There is no cross immunity between "wet" and "dry" type of leishmaniasis.

Wild rodents, such as ground squirrels and gerbils act as reservoirs. The "wet type" is transmitted from these wild rodents through sand flies to men. The Phlebotomus sp. are generally found in the burrows of these rodents and serve as vector of this disease (Vander Hoedon, 1966).

Natural infection of Leishmania tropica has been reported to occur in men and dogs, only two natural cases have been reported in cats (Strong, 1945), in equine (Agnilar et al., 1986). Dogs and rodents act as reservoir of infection. Indian desert gerbil (Meriones hurriance) has also been reported as dominant reservoir host but man is an accidental host (Sharma et al., 1973).

In human beings usually children are found to suffer with ulcerative sore as lesions are usually noticed on their exposed parts of the body upon which a large number of flies feed. In dogs the leishmania sore is found especially on nose, ears, extremities and other hairless parts of the body where the flies can easily bite.

Oriental sore is wide spread disease which occur in various parts of the world. It is prevalent from the shores of Mediterranean to central Asia and the drier parts of central and western India and some parts of China and Africa.

The distribution of the disease in India is not uniform, in some areas it is sporadic where as in other areas it is endemic and is known by local names given to the disease Viz. Delhi boil, Ambala sore, Karnal sore, Bombay sore and Gujrat sore. Sporadic cases have been reported from Fajilka in Punjab and Hanumangarh, Barmer, Jodhpur and Bikaner in Rajasthan.

The endemicity of cutaneous leishmaniasis has been reported from Bikaner (Bhardwaj and Joshi, 1971; Lodha et al., 1971; and Sharma et al., 1973). The incidence of cutaneous leishmaniasis cases shows an increasing trend as observed at the outdoor of skin & V.D. Dept. at P.B.M. Hospital, Bikaner and at the outdoor of Veterinary College Clinic, Bikaner in recent years. Saxenaet al. (1970) have reported the efficacy of unibarb-rine for the treatment in human cases of oriental sore in Bikaner.

Presently, large number of pet dogs of different breeds are reared by human beings at Bikaner city. Apart from this there is also presence of large population of street dogs in Bikaner city. Thus,human population is very closely associated with street and pet dogs, where sandfly is found to be responsible for transmitting the infection of L. tropica among human beings and dogs. Therefore, it was necessary to study about L. tropica infection in men and dogs as regards to epidemiological diagnosis, transmitting vector, treatment and its control. From Public Health point of view this investigation will be helpful in diagnosis of this disease for veterinarians, physicians and pet owners. Looking to public health importance of this disease the proposed investigation is undertaken with the following objectives:-

(i) To find out incidence of L. tropica in dogs in Bikaner city.

(ii) To find out incidence of L. tropica in human beings in Bikaner city.

(iii) Detection and studies on transmitting agent of L. tropica infection i.e. sand fly from habitat of positive cases of men and dogs.

(iv) Cultivation of L. tropica from suspected and positive cases of men and dogs on artificial media (NNN and diphasic media) for confirmation of oriental sore.

(v) Efficacy of 2% aqueous solution of berbarian sulphate in the treatment of human and dogs suffering from leishmaniasis.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The attempts to discover the etiology of cutaneous leishmaniasis (oriental sore) were started by Cunningham (1885) in India. The organism isolated from oriental sore was described as a "Peculiar Parasitic organism in the tissue of a specimen of Delhi boil". Firth (1891) gave the name Sporozoa furunculosa to the parasite causing cutaneous leishmaniasis. Marchand (1904) reported the leishmania species from the spleen of Chinese.

The genus leishmania was established by Ross (1903) to include L. donovani, the heamoflagellate causing Kala-azar. It has been named after its discoverers, Leishman and Donovan, both of whom reported the organism simultaneously. Leishman from London in May, 1903 and Donovan from Madras in July, 1903 (Chatterjee, 1962).

In regards to the etiology of cutaneous leishmaniasis, Wright (1903) of Boston is credited for first complete description of the parasite. He observed the parasite in an Armenian child who had been brought to Massachusetts general hospital and named it Helcosoma tropica. Marzinowsky and Bogroff (1904) discovered a parasite in a sore of boy in Russia and named the organism as Ovoplasma orientale. Luhe (1906) changed the name Helcosoma tropica given by Wright (1903) to L. tropica.Thus,the parasite is named as L. tropica (Wright, 1903; and Luhe, 1906)

Prevalence of cutaneous leishmaniasis

(a) Abroad

The disease is wide spread and occurs in various parts of the world. It is present in the old world throughout the Mediterranean countries, the Middle East and Central Asia. In Africa, it is found around the edges of Sahara including the Sahara base of West Africa and in the highlands of Ethopia and Kenya.

Fergusson and Richards (1910) reported a case in Egypt of granulomatous growth resembling oriental sore. Thomson and Balfour (1910) and Carter (1911) reported cases of non ulcerating oriental sore. The first case originating in the Sudan was discovered in 1911 in Nuba mountains of Kordofan province, the patient was an adult male who had a nodular form of cutaneous leishmaniasis (Archibald, 1911). Neligan (1913) while working in Tehran in Persia, where oriental sore was endemic, discovered leishmaniasis in cutaneous lesions of a dog. Pedroso (1913) noted ulcers on the skin of two dogs which were associated with man, infected with L. tropica. Cutaneous leishmaniasis was described in Ethopia by Martoglia (1913). He and other Italian authors showed its high prevalence over the highlands of Ethopia (Monti, 1937; Poggi, 1937; Cupi and Cattapan, 1942; Barbera and Capuano, 1947; Congolesi, 1948), as cited by Ashford et al. (1973).

Christopherson (1914) reported 2 cases of oriental sore, one from Khartoum province and the other from the Blue Nile province. Yakimoff (1914) found leishmania in cutaneous lesions of a dog in Turkistan.

Gachet (1915) examined 21 dogs in Tehran and found skin lesions due to leishmania infection in 15 of them. Kirk and Drew (1938) also reported 5 similar cases, one from Blue Nile and 4 from Darfur province.

Issayer (1941) noticed a few cases of dogs suffering from cutaneous leishmaniasis. Kojevnikov (1941) reported the prevalence of oriental sore from Turkmenia. Kovziakine (1941) recorded focci of cutaneous leishmaniasis in Eastern Transcaucasia. Incidence of dermal leishmaniasis was recorded in Mexico by Beltran (1944).

Prevalence of cutaneous leishmaniasis has also been reported in some parts of China and Africa (Craig and Faust, 1953) Cahill (1964) described skin lesions in 4 American who had contracted cutaneous leishmaniasis in Blue Nile and upper Nile provinces and Slappendel (1988) had also reported it in Netherland. Pirmez et al. (1988) also reported cutaneous leishmaniasis in canines at American.

(b) India

In India, the distribution of the disease is not uniform, as in some of the places, only sporadic cases of leishmaniasis have been reported while in some other parts of the country it is so prevalent, that these endemic areas could be raced by their local names given to the disease, i.e. Delhi boil, Ambala sore, karnal sore, Gujrat sore and Bombay sore etc. (Sinton, 1925-27).

Avari and Mechie (1924) found ulcers on the ears of a dog positive for leishmania species in Bombay.

A large scale epidemic of oriental sore was recorded in Delhi during 1940, in which approximately 20,000 people suffered in KarolBagh area (Shah,1941). Several reports about the incidence of this disease have been recorded from the district of Aurangabad (Maharastra) and its adjoining area (Young, 1937; Daner and Ahmed, 1943; and Farooq and Cutubuddin, 1945).

Cutaneous leishmaniasis is of two types, dry and wet (Kojevnikov, 1941). The dry type of cutaneous leishmaniasis occurs in the Central, Western and North Western parts of the Indian subcontinent and the noteworthy centres of infection are Lahore, Multan, Delhi, Dera-Ismilekhan etc. (Manson - Bahr, 1951).

Ray (1945) referred to the reports of cutaneous leishmaniasis in dogs from North West frontier province and United provinces in India.

Faust and Russel (1957) mentioned its incidence in dogs of Bombay. Omran (1961) and Lysenko (1971) suggested that cutaneous leishmaniasis or oriental sore was restricted to dry Western half of Indo-gangetic plains, including Arid zones of Rajasthan and Gujrat states. Saxena et al. (1970) tried the efficacy of Uniberberine against oriental sore in men and also reported about occurance of this disease at Bikaner. Its endemicity has also been reported from Bikaner (Bhardwaj and Joshi, 1971; and Sharma et al, 1973). Cases were detected in and

around Bikaner i.e. in Shivbari, Gangashahar and at Nokha in Bikaner district. Lodha et al. (1971) were first to report about the endemicity of cutaneous leishmaniasis in dogs and men in Bikaner.

In India the evidence of the possibility of existence of a zoonotic infection based upon clinico -epidemiological analysis of the cases, was collected in 1973 during the' course of an investigation of an outbreak of cutaneous leishmaniasis in Bikaner as reported by Sharma et al. (1973).

Later,the presence of sporadic cases in the villages in the vicinity of Rajasthan canal area at Tibi and Hanumangarh (ShriGanganagar district) were detected during March 1973 (Sharma et al., 1973). Agarwal (1980), Purohit et al. (1982) and Nirban (1985) also reported about the human cases of cutaneous leishmaniasis in Bikaner.

Morphology :

Wenyon (1926) studied the morphology of L. tropica and L. donovani. Several other workers, subsequently made studies on the morphology of L. tropica.

Strong (1945) studies the morphology of leishmania and according to him they were round or oval bodies averaging 2 to 5 microns in diameter. The nucleus was relatively large and peripherally placed, the kinetoplast was smaller rod shoped or oval and set a tangent to the nucleus.

Craig and Faust (1953) described the morphology of the parasite (Leishmania tropica body) from the host and also from the vector. They also described the morphology of development forms growing in cultures. They reported the two stages in the life cycle of L. tropica, aflagellar or amastigote stage and flagellar or promastigote stage. The aflagellar stage was noticed in man and dog, while the flagellar stage in sandflies and cultures.

The structure of the nucleus and nuclear division in Leishmania tropica have been studied by Sorowri ' (1955). Chang (1956) and Pyne and Chakroborty (1958) studied the electron micro photograph of Leishmania tropica and observed the presence of anonema and basal granule, from which the flagella arises. According to Levine (1961) the various species of L. tropica look alike though there are size differences between strains. Chandler and Read (1961) reported that the parasite was very small, round or oval measuring 1.5 to 4.0 microns in diameter possessing a round nucleus and a rod shaped obliquely placed kinetoplast.

Faust and Russel (1957) also studied the morphology of Leishmania tropica. Mishra (1971) and Farah and Malik (1971) obtained better results by examining histological sections from infected dog tissues.

Reservoirs of cutaneous leishmaniasis :

Pawlowsky (1937-39) reported that in the endemic regions of central Asia, gerbils are important reservoir of L. tropica. About 35 per cent of Phlebotomus papatasii and

Phlebotomus caucasicus caught in their burrows have been found infected and this rodent strain has been transmitted to man.

Latysev and Krjukova (1941) studied the zoonotic importance of cutaneous leishmaniasis with wild rodents in Turkmenia U.S.S.R. They suggested that the large gerbil (Rhombomys opimus) and the Midday gerbil (Meriones meridianus) were the main reservoirs of the causal agents of cutaneous leishmaniasis in the desert and semi desert of Turkmenian U.S.S.R. Mirzyam (1941) also reported gerbils as the reservoirs of leishmania infection in Azarbaidjan U.S.S.R.

Ray (1945) referred cutaneous leishmaniasis in dogs from North-West frontier province and United province of India. Strong (1945) also suggested dog to be principal reservoir of this disease in Baghdad.

Dogs as principal reservoir of this infection have been suggested by several other workers like Neligan, (1913) Pedroso (1913), Yokimoff (1914) and Gachet (1915).

Deane (1956) and Alencar (1958) found an infection rate as high as 27 per cent in dogs at certain focci in Brazil.

Adler and Theodar (1957) were able to find dogs naturally infected with Leishmania tropica in Iran and Iraq. Various desert rodents were described as reservoir hosts for cutaneous leishmaniasis by Chandler and Read (1961) and Levine (1961).

Faust and Russel (1957) mentioned incidence of cutaneous leishmaniasis in dogs of Bombay. Dogs as common reservoir for leishmania have been suggested by Garnham (1965-71) Lainson and Shaw (1948), Shaw and Lainson (1968) indicated the importance of forest rodents as chief reservoirs of cutaneous leishmaniasis.

Dogs as principal reservoir of this disease have also been suggested by Lodha et al. (1971), Mishra (1971) and Bhardwaj and Joshi (1971).

Sharma et al. (1973) reported a 7 per cent infection in Indian desert gerbil (Meriones hurriance) in the vicinity of Rajasthan canal at Tibi, Hanumangarh (Ganganagar District).

Mohan and Suri (1975) carried out studies on Leishmania tropica in India. They isolated cultures of L. tropica from naturally infected gerbils (Meriones hurriance) and sandflies (Phlebotomus salehi) near Rajasthan canal area.

Transmission :

Pressat (1905) and Sergent (1905) suggested that L. tropica is transmitted through sandfly. After ingestion by the sandfly the parasite undergoes development into leptomonads and sometimes leishmania as well.

Patton (1907) in India was the first to be record this type of development in bed bugs fed on kala-azar patients, but Indian kala-azar commision 1925 stressed that inspite of the fact that there was development of flagellates in the bed bugs still

they had nothing to do with the transmission of the infection. The role of sandfly, Phlebotomus papatasii in the transmission of the infection was suggested by Wenyon (1911) and demonstrated by the Sergent brothers with Parrot and Begne (1921). Adler and Theodor (1925) experimentally transmitted cutaneous leishmaniasis to man from Phlebotomus papatasii. Adler and Theodor (1927) used artificially infected sandflies in the transmission experiments of L. tropica by the bite of Phlebotomus sergenti. Adler and Ber (1941) suggested the transmission of L. tropica by the bite of Phlebotomus papatasii. Krjukova (1941) conducted transmission experiment in wild rodents of Turkmenia. Adler and Theodor (1957) showed that phlebotomine sandflies act as transmitting agent for cutaneous leishmaniasis. Vavilova (1960) gave experimental infection of Leishmania tropica in dogs. Berberian (1966) conducted mechanical transmission of leishmaniasis.

Dhanda et al. (1971) conducted survey in six districts of Rajasthan state, they showed that Phlebotomus flies play an important role in transmission of cutaneous leishmaniasis. Sharma et al. (1973) recorded promestigotes forms of the parasite in Phlebotomus papatasii.

Culture :

Rogers (1904) reported that the flagellates developed in sodium citrate solution when spleen suspension having L. donovani was added to it. Nicolle (1908) and Nicolle and Sicne (1908)

obtained culture of leishmania from a case of oriental sore. Nicolle (1908) was the first to cultivate L. tropica on Novy MacNeal and Nicolle's medium in which he observed the flagellar stage of parasite (Craig and Faust, 1953).

Row (1909) and Marzinowsky (1909) also successfully cultivated L. tropica in different artificial media. Wenyon (1911) also cultivated L. tropica. Gingi (1914) gave the optimum temperature range for cultivation of L. tropica and L. donovani as 28^{0} to 29^{0} C and 21^{0} to 22^{0} C respectively.

Nicolle (1925) maintained the strain of L. tropica in culture for 15 years and during this maintenance the material was subcultured for 384 times.

Geiman (1940) cultivated L. tropica satisfactorally in the chorioallantoic fluid of 5 to 9 days old chick embryo.

Experiments to cultivate leishmania were also conducted by Krivkova (1942). Mishra (1971) also tried for the cultivation of L. tropica in tissue culture fluid medium and Novy MacNeal and Nicolle's medium. He was unable to obtain the flagellar stage of the parasite in either of the two media.

Purohit et al. (1982) coducted trials on cultivation of L. tropica from untreated and treated cases of oriental sore. They were able to cultivate L. tropica on Novy MecNeal and Nicolle's media from 20 per cent of human cases which showed

evidence of recent infection. Nirban (1985) cultivated the parasite and revealed that Novy MecNeal and Nicolle's media was better than diphasic media. The flagellar form of L. tropica was observed after 27 hrs of incubation at 22 ^{0}C.

Treatment

Mackif, (1954) used 2 ml of a 1 per cent solution of of berbarian sulphate at weekly intervel for the treatment of cutaneous leishmaniasis. He suggested that three to six injections are usually required for the treatment. Saxena et al. (1970) used uniberbarine successfully against oriental sore in men. Pritipal Singh (1980) tried for comparative study of Metronidizole co-trimoxazole and Berbarian disodium citrate in the treatment of cutaneous leishmaniasis.

MATERIALS & METHODS

3. MATERIALS AND METHODS

TOPOGRAPHY OF BIKANER :

Bikaner city is situated 28° N and 73°, 18° E on a slight elevation about 227 meters above the sea level. At present the municipal council of Bikaner comprises of 45 wards and covers an area of 14,720 acres of land and has population about 4 Lakhs.

The study area comprised of the wall city including new colonies, settlements outside the walled city and rural area newly incorporated in the municipal council of Bikaner. The old city is surrounded by stone wall. It has a number of lofted houses, temples and a massive fort. Majority of these areas, now poorly maintained give the appearance of ruins. In this city there are 5 gate viz. Kote gate, Jassusar gate, Nathusar gate, Sitla gate and Goga gate.

Terrain :

Erskine (1909) described that the three sides of the town are of open type and some cultivation may be possible. The ground on the southern face is intersected by ravines with growth of small shrubs and trees like "JAL" (Salvodera oleoides) and Babul (Acacia arabica). The tree "Khejri" is most commonly found in Bikaner district.

Among the shrubs found in the region mentioned above are of "AAK" (Catotropis procera), PHOG (Calligonum polygonoides) and THOR (Eliphorbia nevulia). Many of these ravines have been excavated into deep caves and are inhabited by cavedwelling birds,

reptiles, rodents and other small animals. Being cool during summer, the ravines offer good day time as resting sites for dogs dwelling in the vicinity and as such make them more prone to the bite of sandflies which live in the burrow of the rodents.

Climate :

Erskine (1909) reported that the climate of Bikaner is dry during summer (May to July) and heat is intense and hot winds blow with great force. Heavy sand storms are common occurrences. Severe cold is experienced during winter. The rainfall in Bikaner in erratic.

The methodology adopted in the present study in recording the epidemiological investigation in men and dogs can be enumerated as below :

[A] To find out incidence of L. tropica in dogs in Bikaner city

A survey of 98 pet dog population of different breeds at Bikaner city and at outdoor clinic of medicine Dept., College of Veterinary and Animal Science, Bikaner was made. A survey of 542 street dogs were also conducted in walled city area of Bikaner.

The details of the pet dogs i.e. breed, sex, age, owner's name, address etc. were recorded. A survey of street dogs was conducted around the area where the human cases of cutaneous leishmaniasis were reported.

During survey 10 pet and 16 street dogs were suspected and these dogs were further examined for cutaneous leishmaniasis at Veterinary Public Health Laboratory, Department of Medicine, College of Veterinary and Animal Science, Bikaner.

[B] To find out incidence of L. tropica in men in Bikaner city

Investigations for cutaneous leishmaniasis in men were made by examining all the cases while attending the outdoor of skin & V.D. Dept. at P.B.M. Hospital, Bikaner. The suspected oriental sore patients were asked to contact once in a week and as such these cases were recorded on every Friday from May to November 1992 which are as follows :- (Table-1).

S.No.	Popular area	Human cases of chronic granuloma presumed to be oriental sore		
		Male	Female	Total
1.	Industrial area, Rani Bazar, Bikaner	04	03	07
2.	Around Pushkarna stadium, Jassusar Gate, Bikaner	09	05	14
3.	CAD Colony, Beechhawal Road, Bikaner	03	01	04
4.	Idgah Bari,Bikaner	05	08	13
5.	South Extension, Pawan Puri, Bikaner	06	11	17
6.	J.N.V. Colony,Bikaner	03	01	04
7.	In side Kote gate, Bikaner	11	04	15
	Total	41	33	74

A brief history was taken from the persons who were showing, typical lesion with pain, itching, fever etc. It was also examined whether ulcer is progressively increasing or decreasing in size.

Past history of chronic non healing ulcer treated by intra lesional injection was obtained. History of close contacts with animals and pets particularly dogs was taken. History of chronic ulcer on dogs residing in surrounding area,if present, was taken. After recording these informations, the person was subjected to clinical examination in presence of good natural day light.

The total number of ulcer present on the body (single or multiple) was also noted. If multiple, whether ulcer was present at the same part or different parts of the body. The site of ulcer was also noted i.e. whether the ulcer was present on the exposed or closed parts of the body. Size and shape of ulcer was measured. Secondary infection in the ulcer, if any was also examined. After noting the extent, site any other details of ulcer, it was classified as nodular type or ulcerative type or non-ulcerated diffused infiltrative type.

Skin smears were prepared from the ulcers of all presumed cases of oriental sore, stained and were confirmed cytologically by the presence of Leishmania tropica bodies. The material from the ulcer of confirmed and suspected cases of leishmaniasis were subjected to culture of the parasite.

A standard questionaire was completed (Appendix I) After recording the name, father's name, age and sex, the person were asked about their occupation and social status.

[C] Vector studies

Sandflies were collected from the houses of the person and dogs presumed to be cases of oriental sore. For this purpose a greasy plate was used and the flies were stored into 70% ethyl alcohol. Fifty seven flies presumed to be sandflies were collected from the different areas of the Bikaner, as shown in Table-2.

For identification and detection of sandfly the studies were conducted in the laboratory with the help of parasitology Dept. College of Veterinary and Animal Science, Bikaner.

Table-2 : Showing areas where the flies were collected

S.No.	Popular area	No of specimen
1.	Around the Pushkarna stadium, Jassusar gate, Bikaner	12
2.	Industrial area, Rani bazar, Bikaner	09
3.	South Extension Pawan puri, Bikaner	08
4.	C.A.D. Colony, Beechhawal Road, Bikaner	07
5.	Sursagar area, Hanuman Hatha, Bikaner	10
6.	Around seemavarti Chhatrawas, Gangashaha road, Bikaner	11
	Total	57

[D] Collection of sample

The samples were collected for two purposes and the same procedure was applied for men and dogs.

(i) Preparation of smear

Contact smears were made directly from the cutaneous lesions after scraping it little bit with the help of sterilized needle. A clean dry greesefree 3"x1" microscopic glass slide was touched over the lesion and then stained with Giemsa's and Leishman's stain.

(ii) For culture

For culture of L. tropica the samples were collected from the cutaneous lesions of positive and suspected cases of L. tropica (L. T. bodies) in men and dogs.

The lesions were curetted with the help of sterilized needle and the oozing was collected into tuberculin syringe and placed directly on NNN and diphasic media under sterilized conditions. Media tubes were placed in B.O.D. incubator at 22 °C for the cultivation of parasite and these were examined daily for the multiplication of parasites.

[E] Staining

For the present study following two staining techniques were used :

(a) Giemsa's staining

(b) Leishman's staining

(a) Method of Giemsa's staining

The staining technique used was :

(i) Films were fixed in methyl alcohol for 3 minutes

(ii) Fixed films were stained in a mixture of 1 part stain and 10 parts of double glass distilled water for one hour.

(iii) Stained films were washed with double glass distilled water.

(iv) Consequently these were blotted and allowed to dry in the air and examined.

(b) Method of Leishman's staining

For preparation of Leishman's stain the powder from the packet was transferred directly into the bottle and dissolved in the solvent by shaking at intervals during 48 hours.

(i) The air dried blood smears were fixed by covering with the stain solution (8-10 drops) for 1 minute.

(ii) After 1 minute, the staining solution on the slide was diluted with twice the number of drops of double glass distilled water and left for a period of 10-20 minutes.

(iii) The slide were rinsed with water and dried by blotting paper or by leaving on edge at room temperature.

(iv) Slides were examined directly under oil immersion of microscope.

[F] Morphology

Examination of stained slides was made under the oil immersion of the microscope. Morphological observations were made both on the intracellular and extra cellular forms of the parasite.

Strong (1945) studied the morphology of Leishmania. According to him they are round/oval bodies averaging 2 to 5 microns. Nucleus is relatively large and placed peripherally, the kinetoplast is smaller, rod shaped or oval and set at a tangent to the nucleus. Craig and Faust (1953) and Faust and Russal (1957) studied the morphology of Leishmania tropica. They reported two stages in life cycle of L. tropica. A flagellar or amastigote stage was noticed in men and dogs, while the flagellar stage in sandflies and during culture of the parasite.

[G] Cultivation

Nicolle (1908) was the first to cultivate Leishmania tropica on Novy, MacNeal, Nicolle's media in which he observed the flagellar stage of the parasite. The same observations were confirmed by Craig and Faust (1953).

Row (1909) and Marzinowsky (1909) also successfully cultivated L. tropica in different artificial media. Nicolle (1925) maintained the strain of L. tropica in cultures for 15 years. During this maintenance the material was subcultured for 384 times.

Cultivation trials of L. tropica were carried out in two artificial media

They were (i) Tobie's Diphasic media

(ii) Novy MacNeal and Nicolles' medium

(i) Preparation of Tobie's diphasic medium

Tobie et al. (1950) described the medium for the cultivation of parasitic protozoa and the name diphasic medium was given as it consisted of a solid phase and liquid phase.

Solid phase :

Bacto-beef (Difco)	1.5 gm
Bacto-peptone (Difco)	2.5 gm
Sodium chloride	4.0 gm
Bacto-agar (Difco)	7.5 gm

All the ingredients were dissolved in 500 ml of double glass distilled water by heating and after cooling, pH was adjusted to 7.2 to 7.4 with NaOH solution and autoclaved at 15 lbs pressure for 30 minutes. This mixture was cooled to a temperature 45 ^{o} to 50 ^{o} C, then whole rabbit blood was added, which was inactivated earlier at 56 ^{o} C for 30 minutes in the proportion of 25 ml of blood to 75 ml of base media. Coagulation of the whole blood was prevented by using heparin.

Liquid Phase

Sterile liquid solution of the following composition was used :

Nacl	8.0 gm
Kcl	0.2 gm
Cacl2.....	0.2 gm
KHP_2O_4.....	0.3 gm
Glucose	2.5 gm

These ingradients were dissolved in 100 ml of double glass distilled water by heating. The mixture was cooled and pH adjusted to 7.4 and autoclaved at 15 lbs pressure for 30 minutes.

Solid media was dispersed in amount of 5 ml into the test tubes. The tubes were kept in slanting position until the base was solidified.

The liquid phase was then added in amount of 2 ml in each tube. The tubes were screw capped tightly and were kept in refrigerator. Strict aseptic conditions were maintained while pouring the media in the tubes.

To avoid bacterial and fungal contamination, antibiotic and antifungal drugs were added.

Antibiotic (Gentamycin)

Two to three drops (40 ug/ml) of gentamycin were added in 50 ml of media with tuberculin syringe.

Antifungal (Tab. Mycostatin)

A tablet of 5 lakh units was dissolved in 10 ml of double glass distilled water and 2 ml or 40 drops of this solution were added in 50 ml of media.

Novy MacNeal and Nicolle's Media (NNN media)

The medium was prepared by mixing agar 14 gm, sodium chloride 6 gm and double glass distilled water 900 ml and boiling the contents of this stock solution, 2 ml was put in each screw capped tube and the tubes were sterilized by autoclaving at 15 lb pressure for 30 minutes. The medium was cooled to 48 ^{o}C. To each of the agar tubes defrinated blood of rabbit equal to 1/3 of the volume of agar in each tube was added. The blood was mixed with the medium and the medium was allowed to cool in slanting position at refrigeration temperature. By cooling the medium at refrigeration temperature the desired amount of water condensation was obtained. The required amount of antibiotic and antifungal drops were added at the rate given before. The tubes were then kept in incubactor at 37 ^{o}C for 24 hours after which they were checked for contamination, if any.

Sixty media tubes, 30 each of Novy MacNeal and Nicolle's and Diphasic medium were used for innoculatin and kept in B.O.D. incubator at 22 ^{o}C for the cultivation of the flagellar stage of the parasite.

[H] Preparation of 2% Berbarian Sulphate aqueous solution

Berbarian sulphate salt ..... 2 gm

Double glass distilled water ....100 ml

Berbarian sulphate salt was triturated in passtle and mortar and dissolved in 100 ml of sterilised double glass distilled water. The prepared solution was filtered through Wattman's filter paper No. 1, and solution was filled into sterilised vials. These vials were autoclaved at 15 lbs pressure for 30 minutes and were stored in refrigerator at 4° to 8° C.

For the treatment of positive cases of leishmaniasis 1 ml solution of berbarian sulphate was injected intradermaly around the lesions at weekly intervals in men and dogs. In all four to eight injections were given for complete recovery of each lesion.

Figure – 1 : Photograph of dog showing cutaneous lesions on facial crest and involvement of both the eye lids.

Figure – 2 : Photograph of dog showing cutaneous lesions on nose.

Figure - 3 : Photograph of dog showing cutaneous lesions on upper lip and around the nose.

Figure - 4 : Photograph of dog showing cutaneous lesions around the nose.

Figure – 5 : Photograph showing the cutaneous lesions on the nose of a dog.

Figure – 6 : Photograph showing the cutaneous lesion around the nose of a dog.

Figure - 7 : Photograph showing L. tropica bodies from a dog (Giemsa 1000x).

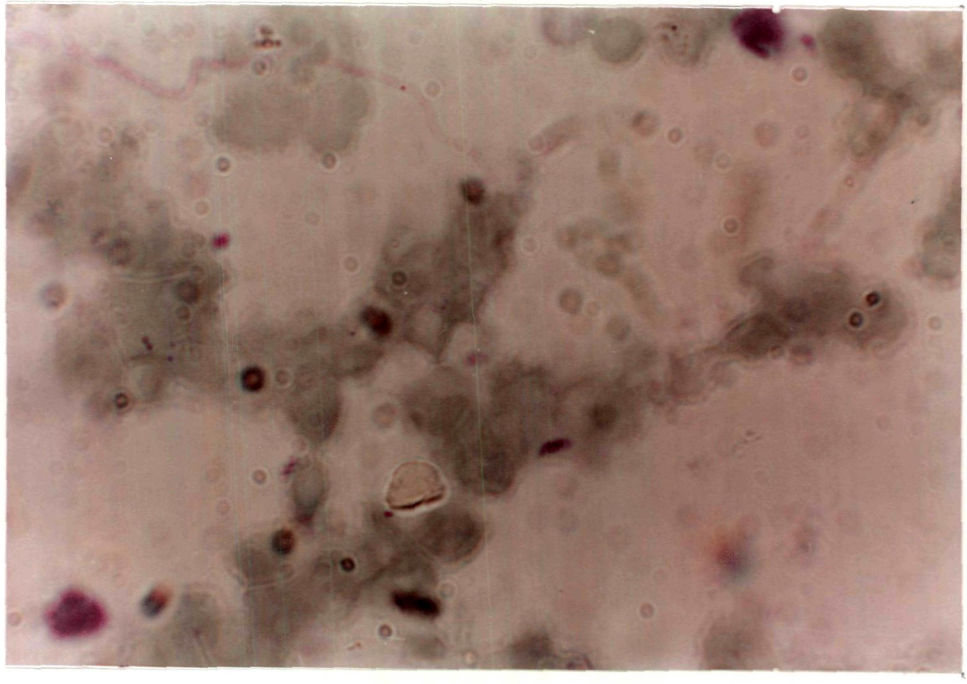

Figure – 8 : Photograph showing mononuclear leucocyte packed with L. tropica bodies from a dog (Giemsa 1000x)

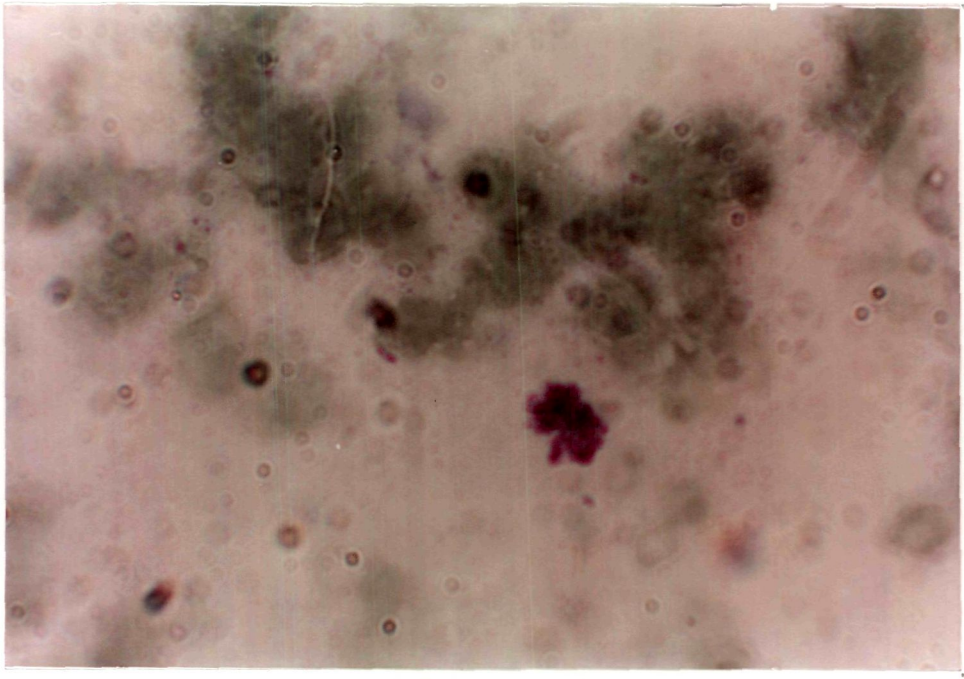

Figure – 9 : Photomicrograph showing mononuclear leucocyte packed with *L. tropica* bodies from human patient (Giemsa 1000x).

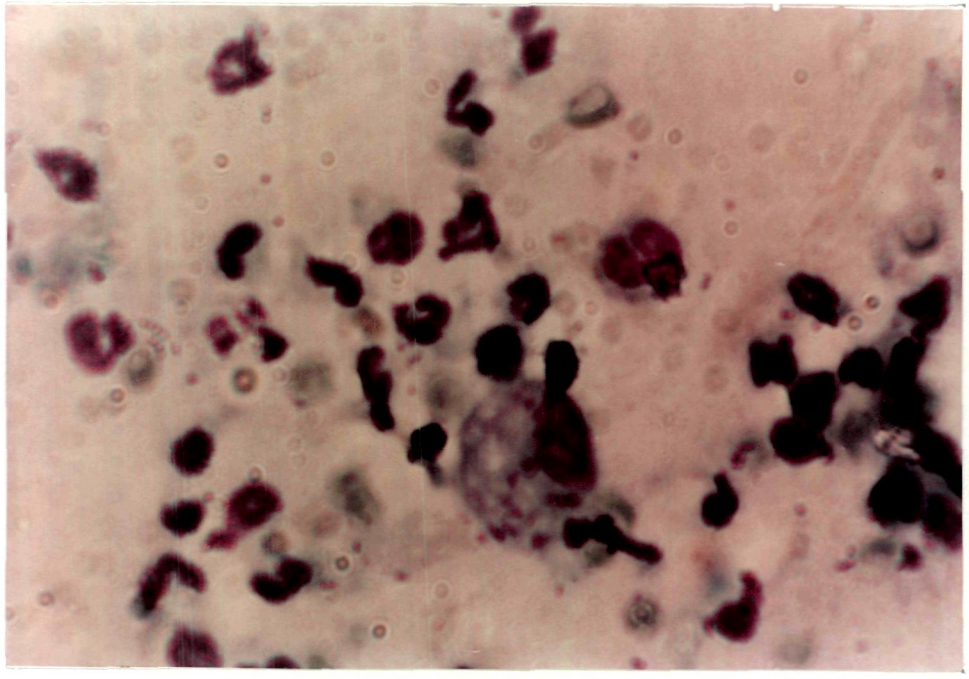

Figure – 10 : Photomicrograph showing mononuclear leucocyte packed with L. tropica bodies from human patient (Giemsa 1000x).

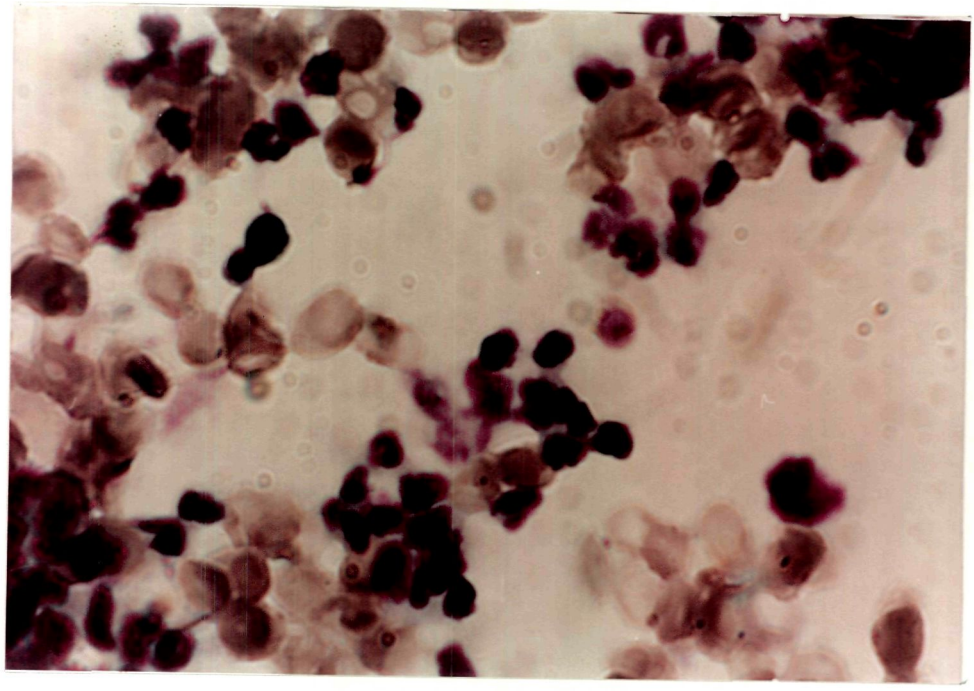

Figure – 11 : Photograph showing the cutaneous lesions on the palm and ankle of a girl.

Figure - 12 : Photograph showing the cutaneous lesion on the nose of a boy.

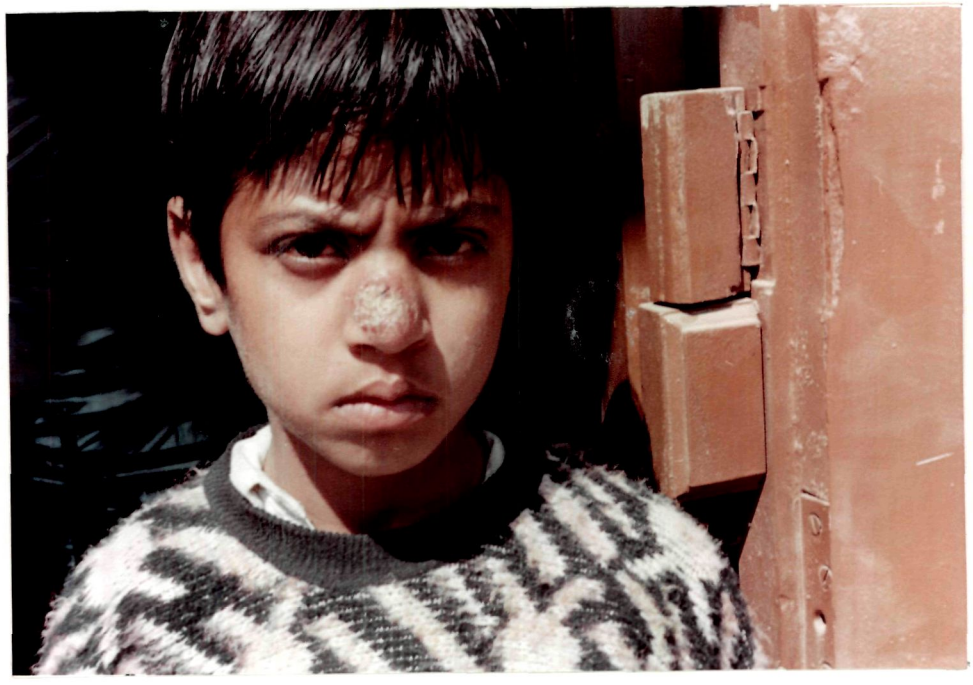

Figure – 13 : Photograph showing the cutaneous lesion on the arm of a woman.

Figure - 14 : Photograph showing multiple Lesions of cutaneous leishmaniasis on the hand of man.

Figure - 15 : Photograph showing the multiple cutaneous lesions on back and hand of a man.

Figure - 16 : Photograph showing multiple lesions of cutaneous leishmaniasis on the chest and leg of a man.

Figure - 17 : Photograph showing the cutaneous lesion with secondary infection on the wrist of a woman.

Figure – 18 : Photograph showing burrows of rat around the houses of human beings suffering from cutaneous leishmaniasis.

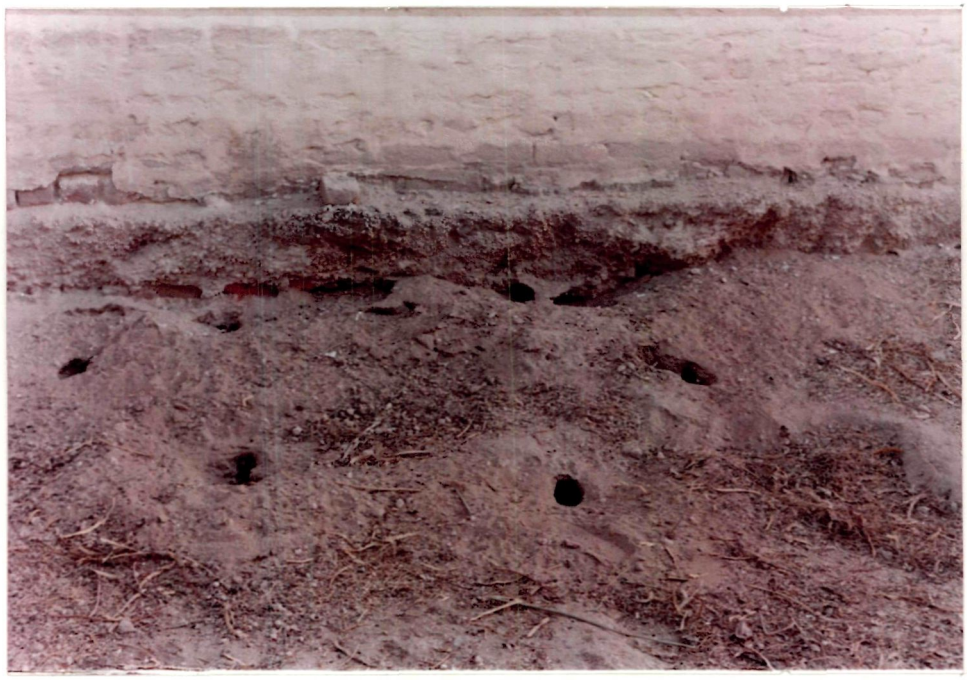

Figure - 19 : A close-up photograph of Phlebotomus species. (sandfly)

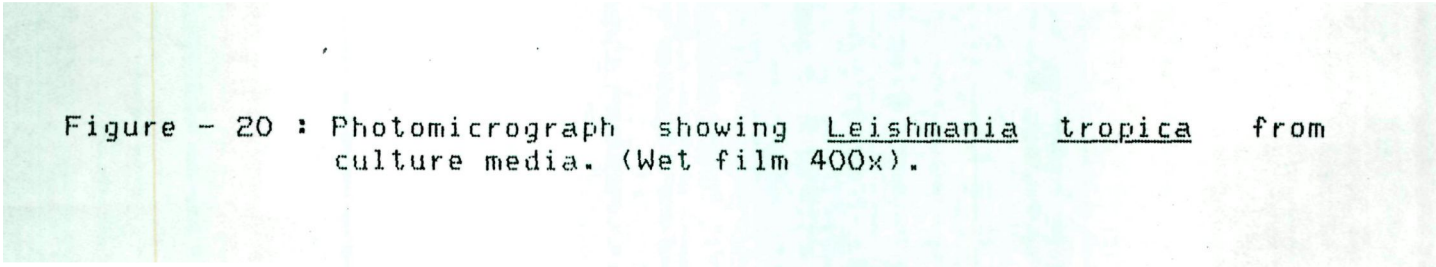

Figure - 20 : Photomicrograph showing Leishmania tropica from culture media. (Wet film 400x).

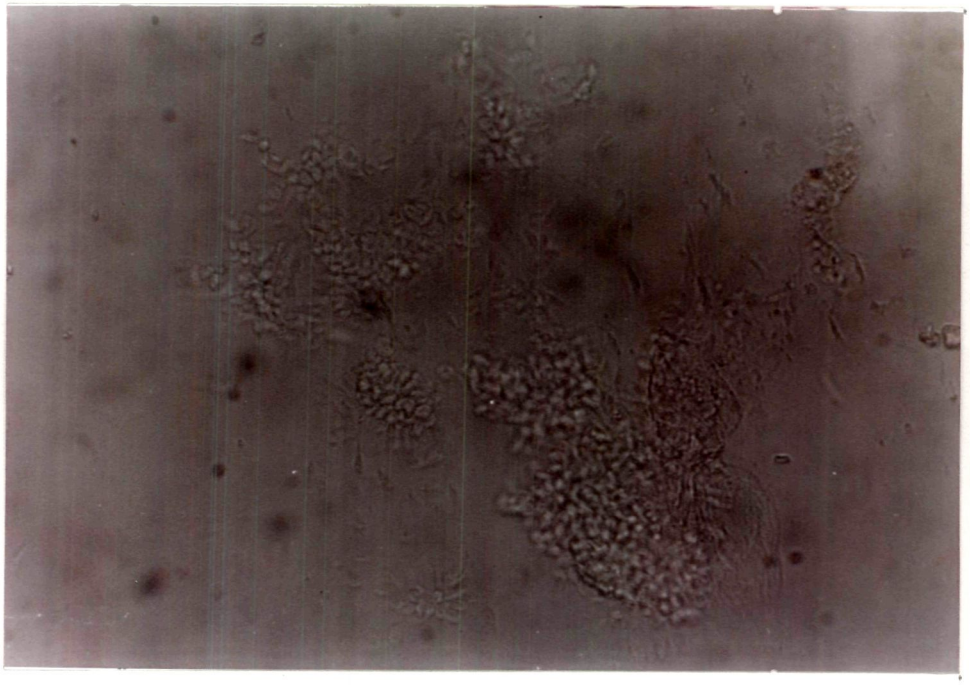

Figure - 21 : Photomicrograph mainly showing promestigote of L.tropica in diphesic culture media (Giemsa 1000x)

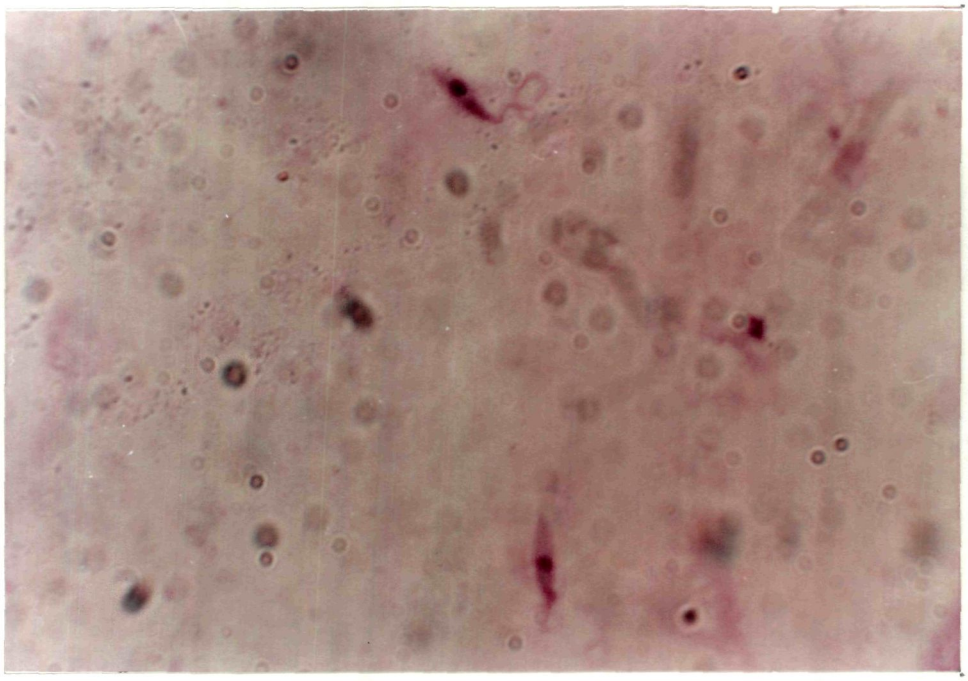

Figure - 22 : Photomicrograph showing promestigotes of L. tropica from diphesic culture media (Giemsa 1000x).

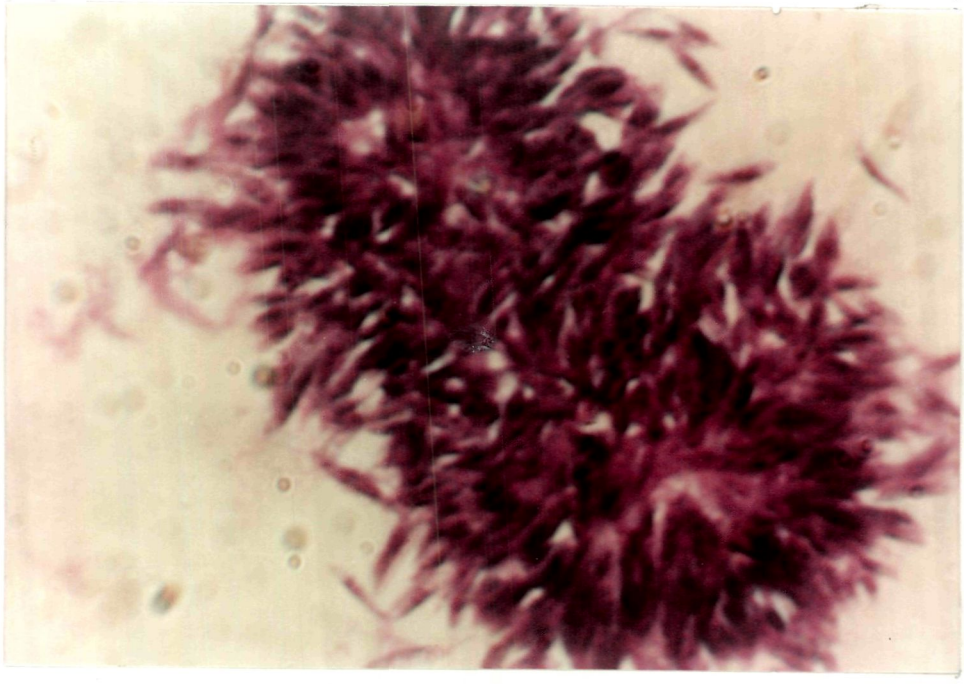

Figure - 23 : Photomicrograph showing promestigotes of L. tropica from diphesic culture media (Giemsa 1000x).

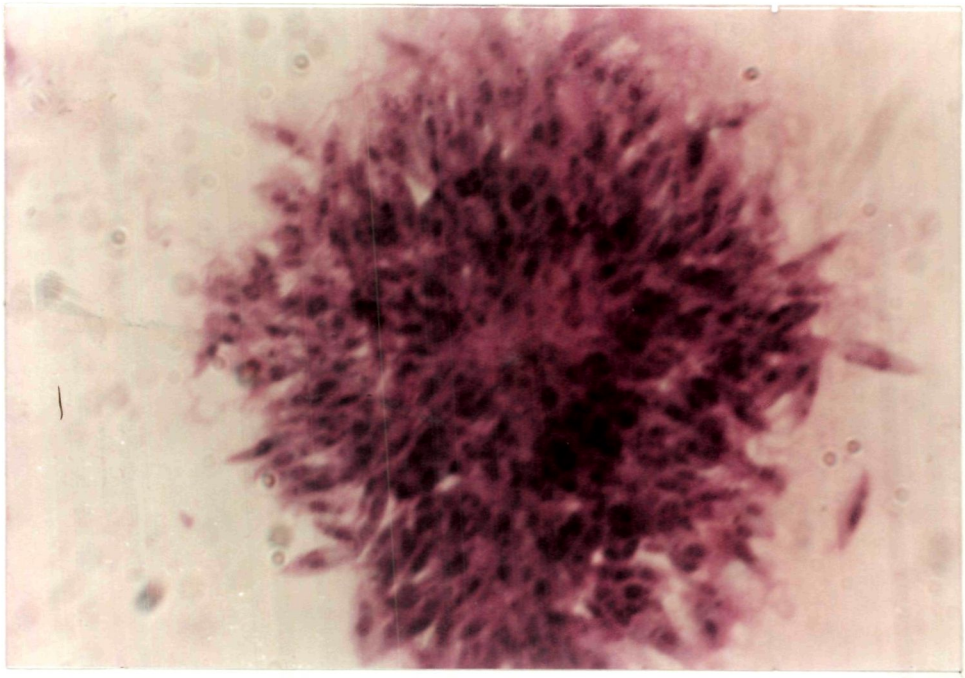

Figure - 24 : Photograph showing the procedure of treatment for the case of cutaneous leishmaniasis of dog (Injecting 2 percent berabarian sulphate solution intralesionally).

Figure - 25 : Photograph showing the procedure of treatment for the case of cutaneous leishmaniasis of man (Injecting 2 percent berbarian sulphate solution intralesionally).

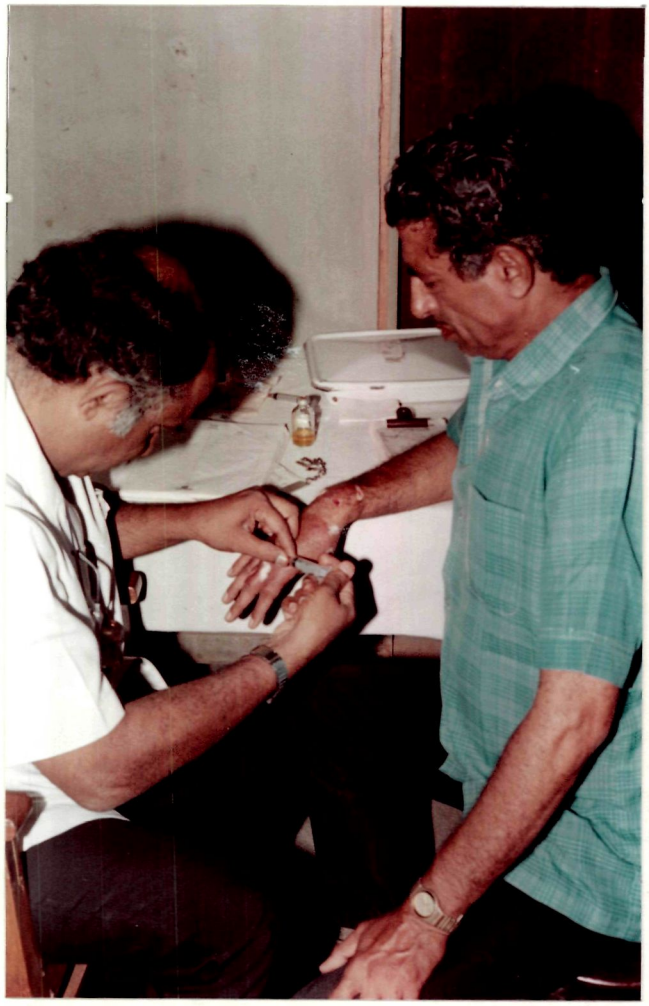

Figure - 26 : Photograph showing the procedure of treatment for the case of cutaneous leishmaniasis of man (Injecting 2 percent berbarian sulphate solution intralesionally).

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RESULTS & DISCUSSION

4. RESULTS AND DISCUSSIONS

A survey of pet dogs, street dogs and human population of Bikaner was conducted to find out the incidence of cutaneous leishmaniasis in all the areas of municipal limits of Bikaner city.

(A) Incidence of cutaneous leishmaniasis in dogs : -

For the purpose of this study, dogs having ulcer on any parts of the body were subjected to clinical examination. Contact smear and blood material for culture from the cutaneous lesions were taken from all the suspected dogs. The prepared slides were stained with Giemsa's stain and were examined for the presence of Leishmania tropica bodies, which could be detected in only 6 cases out of 98 pet dogs and in 11 cases out of 542 street dogs examined and is shown in table-3.

Table-3 : Incidence of cutaneous leishmaniasis in dogs.

S.No.	Types of Dogs	No. of Dogs Examined	Dogs with ulcers	Confirmed cases of cutaneous leishmaniasis	
				No.	per cent
1.	Pet dogs	98	10	06	6.12
2.	Street dogs	542	16	11	2.02

Out of these positive cases of 17 dogs (6 pet dogs and 11 street dogs) 10 were male (3 pet dogs and 7 street dogs) and 7 were female (3 pet dogs and 4 street dogs). Out of these seventeen positive cases of dogs 10 dogs (3 pet and 7 street dogs) were in 1 to 2 years age group.

These 17 cases of dogs were located in various places of Bikaner City i.e. Industrial area, Rani Bazar, Around Pushkarna stadium, Jassusar Gate, C.A.D. colony, Army area, Pawan Puri, Jai Narayan Vyas colony and Inside Kote Gate (Table-4).

Table-4 : Distribution of cutaneous leishmaniasis cases of dogs in Bikaner City.

S. No.	Popular area (Locality of dogs)	Pet Dogs			Street dogs		
		Dogs Examined	Dogs with ulcer	Confirmed cases of oriental score	Dogs Examined	Dogs with ulcer	Confirmed cases of oriental score
1.	Industrial area, Rani Bazar	12	03	01	98	03	01
2.	Around Pushkarka Stadium and Jassusar Gate	18	01	01	95	06	03
3.	Around Seemavarti chhatrawas and Gangashahar road	-	-	-	54	01	01
4.	C.A.D. Colony	08	01	01	-	-	-
5.	South Extension-Pawan Puri	10	02	01	68	01	01
6.	J.N.V. Colony	18	02	01	43	00	00
7.	Army area	20	00	00	-	-	-
8.	Sursagar, Ginnani and Hanuman Hatha	-	-	-	89	02	02
9.	Inside Kote gate	12	01	01	95	03	03
	Total	98	10	06	542	16	11

According to the present observation, cutaneous leishmaniasis in dogs is independent of age and sex as the disease was observed both in male and female dogs of various age groups (Table-5).

Table-5 : Distribution of cutaneous leishmaniasis in dogs according to age and sex.

S. No.	Age group of Dogs	Pet Dogs			Street dogs			Sex			
		Presumed case of O. sore	Dogs with ulcer	Confirmed case of O. sore	presumed case of O. sore	Dogs with ulcer	Confirmed case of O. sore		Pet Dogs	Street Dogs	Total Dogs
1.	Below 1 Yr.	22	02	01	92	01	00	Male	01	00	01
								Female	00	00	00
2.	1-2 Yrs.	32	05	03	121	09	07	Male	01	05	06
								Female	02	02	04
3.	2-3 Yrs.	19	01	01	109	03	02	Male	01	02	03
								Female	00	00	00
4.	3-4 Yrs.	13	01	00	89	01	01	Male	00	00	00
								Female	00	01	01
5.	Above 4 Yrs.	12	01	01	131	02	01	Male	00	00	00
								Female	01	01	02
	Total	98	10	06	542	16	11	Male	03	07	10
								Female	03	04	07

(O. sore = oriental sore)

The cutaneous lesions were having thick indurated margins and were raised giving them the crateriform appearance. There was no suppuration from the lesions but secondary infection with the bacteria was intense. The lesions in one pet dog were very extensive. Lesions were present on the anterior part of the nasal cavity, around nostrils which extended over nasal septum and around the eyes (Figures 1 to 6).

Cutaneous leishmaniasis in dogs is a localised skin infection but in the present study extension of a lesions in the anterior part of nasal cavity in a dog, probably gave false impression of being caused by Leishmania brazilliensis. Although, according to the classification given by Chandler and Read (1961), the cutaneous and muco-cutaneous leishmaniasis are different clinically and the causative agents of two have been differentiated. But, the apperance of muco-cutaneous lesions indicates the presence of Leishmania brazilliensis (Carini, 1911), whereas the geographic distribution in this area indicates the presence of Leishmania tropica. However, morphologically the two organisms are identical (Craig and Faust, 1953). In the present study the organism has been identified as L. tropica, and the prevalence of this infection in this part of Rajasthan has also been reported earlier by Saxena et al. (1970), Lodha et al. (1971) Bhardwaj and Joshi, (1971), Sharma et al. (1973) and Nirban (1985).

This infection has been also reported earlier in dogs from other parts of this country by Avari and Mechie (1924) from Bombay, by Ray (1945) from North West frontier province and United

provinces in India and Faust and Russel (1957) from Bombay. The incidence of this disease was reported to be 6.8 per cent in dogs of Bikaner by Nirban (1985). Presentely, the incidence was recorded to be 6.12 per cent among pet dogs and 2.02 per cent in street dogs. The findings about the incidence among pet dogs are in close agreement with the work of Nirban (1985).The of cases leishmaniasis among pet dogs have been recorded for the first time in this area and this suggests that an increasing trend of such cases will be responsible for high incidence of this disease in human beings of this area and in times to come this disease will emerge as a major zoonotic disease.

The ulcers were irregular in size and shape. Though cutaneous ulcers were present on various parts of the body, majority of them were located on the extremities. Majority of the lesions were less than 12 mm in diameter. A few of them were however as big as 20x56 mm in size which may be due to the fact that the adjacent lesions had coalesced forming bigger ulcers.

Morphology

The impression smears prepared from the cutaneous lesions of the infected dogs, stained with Giemsa's stain and examined under the oil immersion of the microscope were positive for Leishmania tropica (Figure-7).

In the stained smear, the cytoplasma of the parasite was light blue, nucleus appeared to be purple and the kinetoplast a reddish purple. Both intracellular and extracellular forms of the parasites were observed and while making detailed study of

intracellular forms, it was observed that the parasites in the mononuclear leucocytes were heavily packed (Figure-8). These leucocyte packed with parasites attained slightly globular forms and their nuclei shifted to one side but in some of the leucocytes, the nuclei were also found to be in the centre. The nuclei in majority of mononuclear leucocytes were oval or globular in shape. A few of leucocytes, which were excessively packed with Leishmania tropica bodies were visible in ruptured condition.

The extracellular forms of the parasite were generally torpedo shaped, though oval or rounded forms were also present (fig.-7). The nucleus in majority of torpedo shaped parasite were at the broader and while in round and oval forms it was situated towards the periphery. The rod shaped kinetoplast was obliqely packed in the middle of the parasite.

The morphological observations made on Leishmania tropica in the present study were similar to those reported by Craig and Faust (1953), Chandler and Read (1961) Wenyon (1926), Mishra (1971) and Nirban (1985).

(B) Incidence of cutaneous leishmaniasis in human beings :

For the purpose of this study, a clinical non healing ulcer on any part of the body was suspected as a oriental sore (Cutaneous leishmaniasis). Impression smears from the lesions were taken from all the patients and examined for the presence of Leishmania tropica. In present study, 36 cases showed Leishmania tropica bodies out of 74 total cases examined, i.e. 48.6 per cent only (Table-6).

Table-6 : Incidence of cutaneous leishmaniasis in human beings.

S.No.	Presumed cases of oriental sore	Confirmed cases of oriental sore	per cent
1.	74	36	48.6

When prevalence of this disease was examined,a certain interesting factor emerged was that the disease, was primarily confirmed to limited areas of the city (Table-7).

Table-7 : Incidence of cutaneous leishmaniasis in human beings.

S.No.	Popular area	MALE		FEMALE		TOTAL	
		Presumed case of O. sore	Confirmed cases of O. sore	presumed case of O. sore	Confirmed cases of O. sore	Presumed case of O. sore	Confirmed cases of O. sore
1.	Industrial area, Rani Bazar	04	03	03	01	07	04
2.	Around Pushkana Stadium, Jassusar gate	09	07	05	03	14	10
3.	C.A.D. Colony Beechhawal Road	03	00	01	01	04	01
4.	Idgah Bari	05	02	08	04	13	06
5.	South extension, Pawan puri	06	03	11	05	17	08
6.	J.N.V. Colony	03	00	01	01	04	01
7.	Inside Kote gate	11	04	04	02	15	06
	Total	41	19	33	17	74	36

(O. sore = oriental sore)

The incidence rate in this study is entirely based on demonstration of Leishmania tropica bodies in one smear from every human case. Possibility of a variable number of them being oriental sore cannot be ruled out. The disease is wide spread and occurs in various parts of the world. In India, a variable prevalence has been reported. Its endemicity has been reported from Bikaner by Bhardwaj and Joshi (1971), Saxena et al. (1971), Mishra (1971),Lodha et al. (1971) Sharma et al. (1973), Purohit et al. (1982) and Nirban et al. (1985).

In some areas, the disease was so prevalent that their endemic areas gave a local name to the disease, such as Delhi boil, Ambala sore, Karnal Sore and Gujrat sore (Sinton, 1925-27).

A large scale epidemic of oriental sore was recorded in Delhi during 1940, in which approximately 20,000 people suffered in Karol Bagh area (Shah, 1941).

It can be seen in Bikaner that the presence of infected dogs roughly corresponds to the area with the increased prevalence of oriental sore in human being (as shown in map). This suggested a strong association between oriental sore in men and Leishmania tropica body positive ulcers in dogs. The present observation about this disease is in agreement with that of Strong (1945) who also described simultaneous occurence of cutaneous leishmaniasis in dogs and men in Bagdad indicating zoonotic importance of the disease. Lodha et al. (1971) and Sharma et al. (1973) had suggested that dogs principaly acts as reservoir of this infection and similarly this have also been suggested by several other

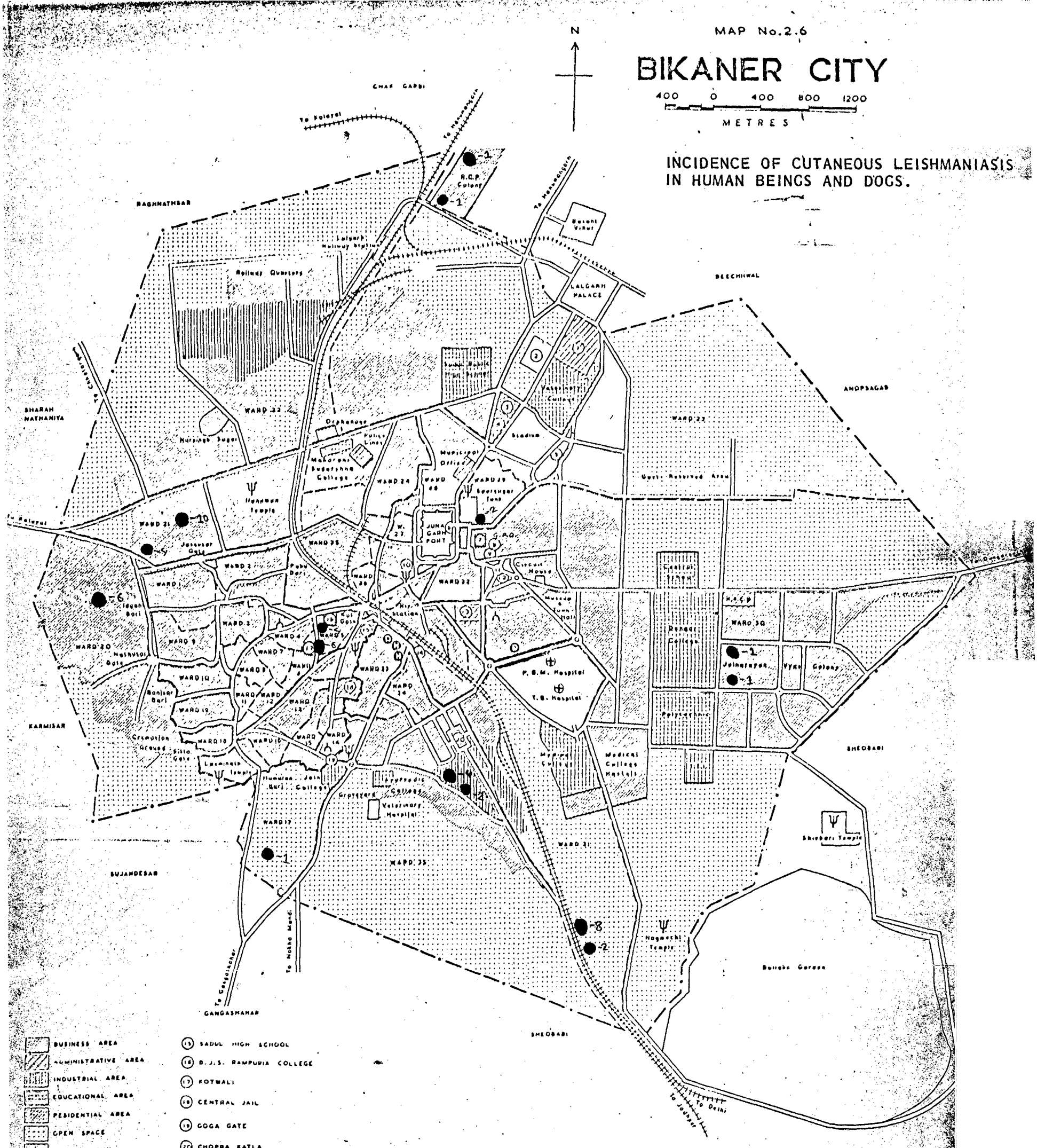
MAP No.2.6

BIKANER CITY

400 0 400 800 1200

METRES

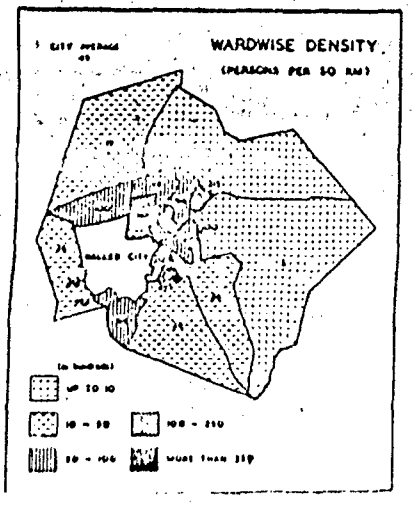
INCIDENCE OF CUTANEOUS LEISHMANIASIS IN HUMAN BEINGS AND DOGS.

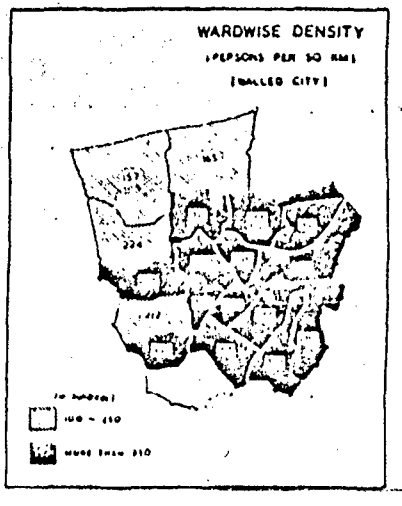


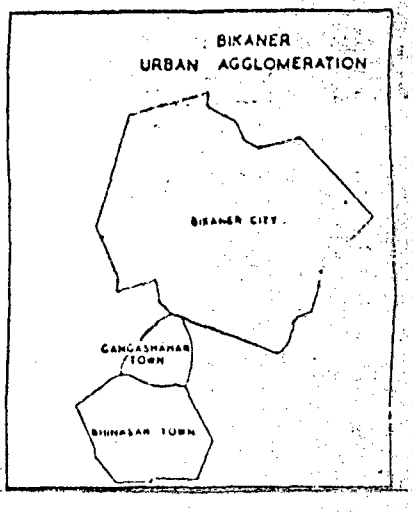
- BUSINESS AREA
- ADMINISTRATIVE AREA
- INDUSTRIAL AREA
- EDUCATIONAL AREA
- RESIDENTIAL AREA
- OPEN SPACE
- TANKS ETC
- TEMPLE
- MOSQUE
- HOSPITAL
- HOTEL
- DHARMASHALA
- G.P.O. GENERAL POST OFFICE
- (1) OFFICE OF THE DIRECTOR SECONDARY EDUCATION
- (2) HOSTEL VETERINARY COLLEGE
- (3) MAHARANI GIRLS SCHOOL
- (4) TEACHERS TRAINING COLLEGE
- (5) GANGA CHILDREN SCHOOL
- (6) SUBAJ CHOWK
- (7) COLLECTORATE
- (8) U.I.T. OFFICE
- (9) GANGA TALKIES
- (10) BAJRATAN BIHARIJI TEMPLE
- (11) PUBLIC PARK
- (12) ZOO
- (13) D.S. OFFICE (RAILWAY)
- (14) DAK BUNGALOW
- (15) SADUL HIGH SCHOOL
- (16) B.J.S. RAMPURIA COLLEGE
- (17) KOTWALI
- (18) CENTRAL JAIL
- (19) GOGA GATE
- (20) CHOPRA KATLA

● → Cutaneous leishmaniasis in Human beings.

● → Cutaneous leishmaniasis in dogs.







workers like Neligan (1913), Pedroso (1913), Yakimoff (1914), Gachet (1915) and Ray (1945).

As per litrature it appears that this disease is endemic for Bikaner, as the incidence has been reported from time to time from this area by Pritipal Singh (1980), by Agarwal (1980) as 0.68 per cent and by Nirban (1985) as 19 per cent. Present investigation revealed the incidence of leishmaniasis to be 48.6 per cent and this indicates that the incidence is increasing as compared to the reports of earlier workers. Probably, the high population of pet and street dogs might be responsible for such a high incidence, where the sandfly can easily be able to transmit the infection among men and dogs.

Morphology

The smears from the cutaneous lesions of human patients after staining with Giemsa's stain when examined under oil immersion, revealed the presence of Leishmania tropica bodies within the monocytes, the organism was in the amastigote form, the cytoplasm appeared as a pale blue with single nucleus and a rod shaped kinetoplast with reddish purple colour (Figures 9,10). In same stained smears, parasite appeared outside the host cells, but it can be assumed that this has occurred due to the rupture of the host cells. The morphological observations recorded in the present study are similar to those reported earlier by Craig and Faust (1953), Chandler and Read (1961), Wenyon (1965), and Mishra(1971).

Age wise distribution : -

Age-wise incidence was determined and presented in table-8.

Table-8 : Distribution of cutaneous leishmaniasis in human beings according to age and sex.

S.No.	Age group	Presumed cases of oriental sore	Confirmed cases of oriental sore	Sex	
1.	Below 20 Yr.	42	23	Male	15
				Female	08
2.	Above 20 Yr.	32	13	Male	04
				Female	09
	Total	74	36	Male	19
				Female	17

Persons below 20 years of age showed a high incidence of this infection (23 out of 36 cases). It reveals from the present findings that this disease has a strong predilection for the young age group. Mishra (1971) and Nirban (1985) have also suggested that younger children play often with dogs and this is probabely responsible for increased predilection of this disease in younger age group. Similar observations have been made by Daner and Ahmed (1943), Farooq and Cutubuddin (1945), Lodha et al. (1971) and Sharma et al. (1973). This may also be one of the reasons that persons having ulcers in their earlier life rarely suffer from this disease in later life as immunity appears to last for longer period and similar observation has also been recorded earlier by Farooq and Cutubuddin (1945).

Sex wise distribution

Sex-wise incidence of human cutaneous lishmaniasis was determined and is presented in table-9.

Table-9 : Sex wise distribution of human cutaneous leishmaniasis.

S.No.	Sex	Presumed cases of oriental sore	Confirmed cases of oriental sore
1.	Male	41	19
2.	Female	33	17
	Total	74	36

In the present study, a total of 74 persons were examined, out of which 41 were male and 33 were female (Table-9). Out of total 36 positive cases, 19 were male and 17 were female , giving ratio of 1.1 : 1. Agarwal (1980) recorded the infection in male and female human being with a ratio of 1.3 : 1. Presently, our results are also in close agreement with the findings of Agarwal (1980).

Number of ulcers on body

Number of ulcers on the body of human patients was determine and presented in table-10.

Table-10 : Showing no. of ulcers on body of human beings suffering from cutaneous leishmaniasis.

Sex	Singuler ulcer		Multiple ulcer		Total	
	Presumed cases of O. sore	Confirmed cases of O. sore	presumed cases of O. sore	Confirmed cases of O. sore	Presumed cases of O. sore	Confirmed cases of O. sore
Male	30	14	11	05	41	19
Female	16	11	17	06	33	17

(O. sore = oriental sore)

In all,there were 41 presumed cases of oriental sore in male group, and out of these 30 showed single ulcer, while 11 showed multiple ulcers, but only 14 (Figure-12) and 5 (Figure-14,15 & 16) cases were confirmed respectively by skin smears examination. Amongst 33 presumed cases in female, 16 showed single ulcers, and 17 showed multiple ulcers, but only 11 (Figure-13) and 6 (Figure-11) cases were confirmed respectively by skin smear examination.

In some patients, multiple ulcers were seen where the adjacent ulcers had coalesced together and formed a big ulcer (Figure-16). Secondary infection could be detected mostly in cases of multiple ulcers. It was probably due to scratching and itching of ulcers and the activities of the ubiquitous house flies (Ashford et al, 1973).The results of the present study are similar to that of Chandler and Read (1961), as they have also observed multiple ulcers in majority of the human cases.

Distribution of lesions on body : -

In present investigation, almost all patients showed cutaneous lesions on their exposed parts of the body and these can be observed in figures 11 to 17. The distribution of the lesions are depicted in table-11.

Table-11 : Distribution of cutaneous lesions on different parts of the body in human beings.

S.No.	Part of the body	Presumed cases of oriental sore	No. of confirmed cases.
1.	Face	12	08
2.	Foreasm	19	12
3.	Hand	15	06
4.	Feet	09	03
5.	Leg	13	05
6.	Abdomen	06	02
	Total	74	36

Out of 36 patients, 12 had cutaneous leisons on forearm, and 8 on face, 6 on hand, 5 on leg, 3 on feet and 2 on abdomen. The present result supports the hypothesis that this disease is transmitted by a bite of sandfly, which naturally bites on exposed parts of the body. Similar pattern of distribution of ulcers was also described by Wright (1903), Daner and Ahmed (1943), Farooq and Cutubuddin (1945), Chandler and Read (1961), Lodha et al (1971), Sharma et al (1973), Agarwal (1980) and Nirban (1985).

Duration of lesions :

The incidence of positive cases of oriental sore coming at outdoor of skin and V.D. Department at P.B.M. Hospital, was high with the duration of ulcer between 1 to 2 months as

presented in table-12. As out of 36 confirmed cases, 25 cases had lesions of less than two months duration.

Table-12 : Showing duration of lesions in human beings.

S.No.	Duration in Months	Presumed cases of O. sore	Confirmed cases of O. sore
1.	Less then 1 month	14	03
2.	1 to 2 months	31	22
3.	3 to 4 months	12	07
4.	5 to 6 months	11	04
5.	Above 6 months	06	00
	Total	74	36

(O. sore = oriental sore)

This is probably due to the fact that the older cases naturally heal, and similar trend of infection has also been observed earlier by Agarwal (1980).

Size of lesions :

Most of the ulcers, 25 out of 36 were having a diameter upto 2 cms as depicted in table 13. One case had diameter of more than 6.0 cm. Similar observations have been made by Lodha et al. (1971), Mishra (1971), Agarwal (1980) and Nirban (1985).

Table-13 : Showing size of lesions in human being suffering from cutaneous leishmaniasis

S.No.	Approximate diameter in cm	Presumed case of O. sore	Confirmed cases of O. sore
1.	up to 2 cm	38	25
2.	2 to 4 cm	16	06
3.	4 to 6 cm	11	04
4.	More than 6 cm	09	01
	Total	74	36

(O. sore = oriental sore)

Oriental sore with Secondary infection :- (Table-14)

In males, out of 14 cases of single ulcer, 4 were associated with secondary infection and out of 5 cases of multiple ulcers, 2 were associated with secondary infection. In female 4 cases were proved to be of secondary infection (Figure-16). In female, out of 11 cases of single ulcer, 2 were associated with secondary infection, and out of 6 cases of multiple ulcers, 2 were associated with secondary infection (Figure-17).

Table-14 : Showing oriental sore with secondary infection.

Sex	Single ulcer			Multiple ulcer			Total		
	Presumed cases of O. sore	Confirmed cases of O. sore	Secondary infection	Presumed cases of O. sore	Confirmed cases of O. sore	Secondary infection	Presumed cases of O. sore	Confirmed cases of O. sore	Secondary infection
Male	30	14	04	11	05	02	41	19	06
Female	16	11	02	17	06	02	33	17	04

(O. sore = orienatl sore)

(C)Vector studies :

Collection of sandflies was made in the different areas of Bikaner i.e. from the surrounding areas and houses of the persons and dogs owners, who were showing the positive lesions for oriental sore (Figure-18). In all, 57 specimens which were collected from the different areas only 17 specimens were confirmed as Phlebotomus species of sand fly (Figure-19).

Sandflies are very small brownish hairy flies which can generally be distinguished at first sight by their nearly erect rather narrow wings and slender bodies as reported by Smart (1956) and Whitelock (1960).

Phlebotomus species acts as a vector for L. tropica infection as it has been suggested by Adler and Theodor (1957). Chandler and Read (1961), and Dhanda et al. (1971) have recorded role of Phlebotomus species of fly in transmission of oriental sore after survey in six districts of Rajasthan and further, Sharma et al. (1973) recorded promastigote forms of this parasite from the Phlebotomus papatassi species of sandfly captured in Bikaner area. The present investigation, further confirms that the presence of flies which were captured from the areas of affected cases of oriental sore are definately responsible for the incidence of this disease in men and dogs in Bikaner.

(D) Culturing :

For the cultivation purpose of L. tropica, Novy, MacNeal and Nicolle's and Tobie's Diphasic media tubes were inoculated with freshly collected innoculum from the cutaneous lesions of suspected and positive cases of dogs and human beings. These tubes on incubation, in B.O.D. incubator at 22 °C, revealed the presence of Leishmania tropica for varying period of time. However, the flagellar or the promastigotes form of the parasite appeared after the third day onwords in both the types of culture media tubes (Figures 20 to 23).

On examination under oil immersion, after staining with Giemsa's stain, the promastigotes appeared long and wide. The flagella were half the size of the parasite. In the present study, diphesic media was found to be superior for cultivation of L. tropica. It was further observed that even for the suspected cases of oriental sore, the parasite was easily cultivated from the patients whose impression smears were found to be nagative for LT bodies. Subsequently, the parasite could very easily be cultivated on subculture in diphasic media tubes.

Purohit et al. (1982) and Nirban (1985) also cultivated the parasite from cases of human cutaneous leishmaniasis. Mishra (1971) could not able to cultivate this parasite from the positive cases of dogs on cultur media. From the results of present study, it can be concluded that the diphasic media can very well be used for culture of L. tropica, and it can serve as good tool for the confirmation of suspected cases of men and dogs.

Treatment of oriental sore :

In the present study, the treatment trial was under taken for oriental sore. For treatment, 1 ml of 2 per cent berbarian sulphate solution was injected intradermally at weekly interval in each lesion of human beings and pet dogs (Figures 24,25 & 26) and the results are presented in tables 15 and 16. Five to six injection of berbarian sulphate were needed for the healing of tensions.

Table-15 : Showing observation for treatment of cutaneous leishmaniasis in human beings.

S.No.	Total No. of injection for healing of lesions (2 per cent berbarian sulphate, 1 ml)	No. of cases recovered after injection.
1.	4 injections	01
2.	5 ''	07
3.	6 ''	17
4.	7 ''	05
5.	8 ''	02
6.	irregularly treatment	04

Table-16 : Showing observation for treatment of cutaneous leishmaniasis in pet dogs.

S.No.	Total No. of injections for complete healing of lesions (2 per cent berbarian sulphate, 1 ml)	No. of cases recovered after injection.
1.	4 injections	01
2.	5 "	04
3.	6 "	00
4.	7 "	01
5.	irregularly treatment	00

Earlier Mackif et al. (1954), Saxena et al. (1970), Pritipal Singh (1980) and Purohit et al. (1982) have emphasised on the efficacy of berbarian salt for the treatment of cutaneous leishmaniasis cases occurring in men. The present study also revealed that berbarian sulphate is effective against oriental sore cases of human being and dogs of this area and the results are in agreement with the work of earlier authors.

SUMMARY

5. SUMMARY

For public health point of view cutaneous leishmaniasis is a very important zoonotic disease occurring in Bikaner, Rajasthan. It is a metazoonotic disease caused by a protozoan parasite Leishmania tropica. A sample survey was undertaken to determine the incidence of cutaneous leishmaniasis and degree of its variation in different areas of Bikaner city. The survey was conducted upon street dogs, pet dogs brought at canine clinic of Veterinary College, and human cases which came at out-door of skin and V.D. Department, P.B.M. Hospital, Bikaner.

Attempts to cultivate Leishmania tropica in various artificial media were made. Vector studies were also conducted in the houses and surrounding localities of the human and dog patients which showed a positive skin smear for Leishmania tropica.

Presently, 542 street and 98 pet dogs of different breeds from various areas of Bikaner were examined. Cases of cutaneous leishmaniasis were only restricted where the human patients of oriental sore were reported. Out of 542 street dogs examined, 16 were having ulcers, but only 7 were proved to be positive on skin smear examination and the remaining 4 were proved positive only on cultural examination and overall incidence was found to be 2.02 per cent. Out of 98 pet dogs examined, 10 were having skin ulcers but only 4 were proved to be positive on skin smear examination and 2 were proved to be positive only on

cultural examination and the overall incidence was found to be 6.12 per cent.

Cutaneous leishmaniasis in dogs is an incidious late ulcerating, dry type of disease. It is a localised skin infection. The disease is independent of age and sex. Single and multiple lesions were observed at different parts of the body but majority of them were located on the extremities. Both intra and extra cellular forms of L. tropica were observed in contact smear prepared from the cutaneous lesions. The host cells were mononuclear leucocytes, which in certain cases were literally packed with the parasite.

In case of human beings, 74 patients having chronic ulcers were examined, but only 36 (48.6 per cent) were proved to be the case of oriental sore on skin smear examination. Areas around Pushkarna Stadium, Jassusar Gate and South Extension, PawanPuri were found to be the most affected for oriental sore.

Out of total 36 proved cases of oriental sore of human beings, 19 were male and 17 were female cases (Male female ratio 1.1 : 1). Persons below 20 years of age showed high incidence of leishmaniasis (23 out of 36). Ulcers of one to two months duration were found in most of the cases (22 out of 36).

Leisons were mainly confined to exposed parts of the body. It was further observed that it is a localised skin infection, as not a single case of Leishmania tropica body was observed in general circulation.

Culture studies revealed that the parasite can grow in both the media i.e. Novy MacNeal and Nicolle's media (NNN media) and Diphasic media. For the cultivation of L. tropica Diphasic media was found to be superior than NNN media.In culture media the flagellar form of Leishmania tropica was appeared on third day and onwards when cultivated in B.O.D. incubator at 22°C.

For the treatment, both in dogs and human beings 2 per cent aqueous solution of berbarian sulphate was used. Around the each lesions of 2 per cent berbarian sulphate was injected intradermally at weekly interval. Five to six injections were enough for proper healing of lesions. Presently, berbarian sulphate was found to be suitable for the treatment in human and dog cases of oriental sore.

The present investigation strongly suggests the zoonotic importance of this disease because of high incidence of this disease is occurring in the dogs. In this area dogs act as active carrier for the transmission of this disease to human being through the Phlebotomus species of fly which acts as a vector in this disease .

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APPENDIX

APPENDIX - I

Proforma for the Study of prevalence of cutaneous leishmaniasis

1. Name
2. Age
3. Sex
4. Address
5. Social status
6. Occupation
7. House Room Family size
8. History of present illness

   Ulcer-Painful/Painless

   Fever/Duration/Itching

   Other manifestations :
9. History of past illness:

   Past history of chronic non-healing ulcer treated by intra lesional injection :
10. History of contact with animals :

   General Physical examination:

   Local examination :

   (a) No. of ulcer

   (b) Site

   (c) Size

   (d) Shape

   (e) Local oedema

   (f) Induration

   (g) L. tropica body (positive/negative)