INTRODUCTION

Cutaneous Leishmaniasis (CL) is caused by haemo-flagellate genus *Leishmania*, which infect domestic and wild mammals, including human being (Gradoni et al., 1999). Cutaneous Leishmaniasis in Pakistan has been a burning seasonal problem in the Canine and Human being. Present study was therefore conducted to generate baseline data on Leishmaniasis in three districts of Sindh-Province, Pakistan (Al-Samarai et al., 2009; Aneela et al., 2011; Bari et al., 2011). Leishmaniasis is an endemic disease in certain areas of Pakistan and infection transmitted via the bite of phlebotomine sandfly and presence of its vector is associated with occurrence of cases in endemic areas, (Al-Zahrani et al., 2004). Cutaneous Leishmaniasis has emerged as a challenging infectious disease in the form of new outbreaks in different areas of Sindh-Province, Pakistan and Balouchistan, (Cortes et al., 2004; Kakar, and Suleman. 2004). This disease is wide spread and may cause serious health problems in communities through Mediterranean regions and the Middle East, including Pakistan, (Brooker et al., 2004). There are estimated 12 million cases world wide and 1.5 million new cases of Cutaneous Leishmaniasis (CL) are added each year suggested from tissue samples, (Parviz et al., 2008; Moradi., 2009). The disease affects all ages of human beings including children. This study was conducted to detect Leishmaniasis through clinical way and confirmed by Polymer-
ase Chain Reaction (PCR) in suspected tissue samples.

MATERIALS AND METHODS

STUDY AREA

Tissue samples were collected from Hyderabad were (18), Dadu (41), and Jamshoro (10) total 70 samples were collected of patients from different health centers and clinics of challenged districts in Sindh-Province, Pakistan for detection of Cutaneous Leishmaniasis (CL) via clinical diagnosis followed by Polymerase Chain Reaction (PCR).

SAMPLE SIZE

Tissue samples of human beings between 08-60 years of ages were collected from ulcerative areas washed thoroughly with 70 percent alcohol and size were 0.3-0.5 cm. The tissue samples were brought at Molecular & Parasitology Laboratory, Sindh Agriculture University, Tandojam and kept them into bijou bottles that containing 3-5 ml of 70 percent alcohol to keep tissue for further processing.

DNA EXTRACTION

DNA was extracted through Qiagen column kit. Protocol was followed according to manufactures guide.

DNA PURIFICATION PROTOCOL

Name of kit: DNeasy Blood & Tissue Kits.

DNA QUANTIFICATION

DNA was quantified on Nano drop Spectrophotometer, (ND-1000, Thermo, and Scientific, USA) at 260/280 nm wave length.

PCR CONDITIONS

The following thermal conditions as initial denaturation at 94°C for 5 minutes followed by 30 cycles including denaturation at 94°C for 35 seconds annealing at 60°C for 35 seconds, extension at 72°C for 45 seconds and final extension at 72°C for 5 minutes were used. At the end 5µl of the reaction mixture was analyzed by 2% Agarose gel electrophoresis. These primers were evaluated with Leishmania standard species including Leishmania, (MCAN/IR/97/ LON 490), Leishmania major, (MHOM/IR/75/ER) and Leishmania tropica, (MHOM/IR/04/Mash 10).

PRIMERS

Specific oligonucleotide primer as forward (5’-CAA- CACGCCGCTCC TC T-3) and Reverse primer (5’-AAA CAA AGG TTG TCN GGG-3’) were used.

RESULTS

Detection of Leishmania tropica and Leishmania major in human beings via polymerase chain reaction (PCR) in districts Dadu, Hyderabad and Jamshoro.

Figure 1: Reveals that, out of 61 Leishmania positive tissues 58.57% tissues were found positive for Leishmania tropica and 41.43% tissues were found positive for Leishmania major

Prevalence of cutaneous leishmaniasis on different body parts in districts Dadu, Hyderabad and Jamshoro.

Figure 2: Indicates that, the distribution of infection according to the body parts the 51.42% victims had lesions on the faces where as 22.85% had lesions on hands 18.57% had lesions on legs and 7.14% lesions were observed on the abdomen.

Gel documentation showing positive bands for Leishmania tropica at 620 base pairs.

Figure 3: Showing positive bands for Leishmania tropica at 620 base pairs. Plate.1 is a Gel documentation which reveals that, Leishmania tropica bands appeared at 620 base pairs.
Ongoing research has shown that Leishmania species are endemic in various regions of the world, with significant implications for public health. The identification and classification of these species are crucial for understanding the epidemiology and development of effective treatment strategies. DNA-based techniques, such as Polymerase Chain Reaction (PCR), have been instrumental in the accurate identification of Leishmania species, providing insights into the geographic distribution and species-specific transmission patterns.

During the present study, cutaneous Leishmaniasis was detected in 61 (87.14%) out of 70 samples collected from three districts in Sindh Province. The predominant species identified were Leishmania tropica and Leishmania major. The infection rate was highest on the face, followed by the hands and legs. The difference in infection rates may be attributed to geographical location and strain zoogeography, as suggested by previous studies (Grazielley et al., 2012; Mormano et al., 2013).

The study also highlighted the importance of medical centers and facilities, where patients from other districts seek treatment. This influx of patients can increase the prevalence of Leishmaniasis in areas that are not endemic but have access to medical facilities. The establishment of Leishmaniasis is associated with that of the establishment of sandfly population, as observed in Sindh Province of Pakistan (Fallah et al., 2011; Ershadi et al., 2012; Seray et al., 2013).

The results of this study underscore the need for continued monitoring and control measures to prevent the spread of Leishmaniasis. The development of species-specific diagnostic tools and effective treatment regimens is crucial for managing the disease in endemic regions. The integration of public health strategies and medical facilities is essential to address the challenges posed by Leishmaniasis, ensuring the well-being of the population.
spreading mostly found in rural areas of Sindh Province and their surrounded areas. The disease caused by the intracellular protozoan parasite *Leishmania* represent a major global health problem and World Health Organization (WHO) classified neglected tropical disease. Nearly 10% of the world’s population is at risk of acquiring a form of Leishmaniasis. Worldwide it is estimated that there are 12 million active cases of Leishmaniasis, with 2 million new cases occurring each year. Among parasitic infections, this disease is responsible for the highest number of disability adjusted life years a measure of health burden after malaria.

**ACKNOWLEDGMENTS**

The authors highly acknowledged the Department of Veterinary Microbiology, Faculty of Animal Husbandry & Veterinary Sciences Sindh Agriculture University Tandojam, Department of Veterinary Parasitology, Faculty of Animal Husbandry & Veterinary Sciences, Sindh Agriculture University Tandojam and Central Veterinary Diagnostics Laboratory (CVDL) Tandojam, Sindh-Pakistan for providing research facilities to carry out some part of this work and their inputs and guidelines during this manuscript.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

**AUTHORS CONTRIBUTION**

Muhammad Ismail Qureshi was conducted the research work whereas, Abdul Ahad Soomro was the advisor. Muhammad Ismail Qureshi wrote the manuscript.

**REFERENCES**
