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SHORT COMMUNICATION

Visceral leishmaniasis from a non-endemic Himalayan region of Nepal

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Abstract

Visceral leishmaniasis (VL) is endemic to the southern plains of Nepal. Here, we report the first case of VL from a non-endemic Himalayan region of Nepal. The patient presented with a history of high-grade fever, splenomegaly, and anemia but had not traveled to a VL-endemic region. Visceral leishmaniasis was diagnosed following microscopic detection of the *Leishmania* species amastigote in a bone marrow aspirate, positive result for the rK39 test, and further validation by nested polymerase chain reaction (PCR). The patient was treated with 5 mg/kg liposomal amphotericin B and was clinically improved upon discharge. Our result suggests that VL is expanding towards non-endemic regions of Nepal, and it should therefore be considered that VL surveillance systems be strengthened, particularly for non-program districts and VL be included as a differential diagnosis in febrile illnesses.

Keywords Visceral leishmaniasis · Himalayan region · PCR · Nepal

Introduction

The intracellular protozoan parasite, *Leishmania*, is the causative agent of leishmaniasis, which has been reported from 98 countries and is responsible for about 0.7 to 1 million reported annual cases worldwide (Alvar et al. 2012). Second to malaria, it is the most common cause of mortality due to parasitic infections, with 20,000 to 30,000 deaths annually (Alvar et al. 2012). Children below 15 years of age have been reported as the major victims of the disease (Hotez et al. 2006; WHO Leishmaniasis Fact Sheet 2017). Among the various forms of leishmaniasis, visceral leishmaniasis (VL), commonly called kala-azar, is the most common in the Indian subcontinent (Alvar et al. 2012). VL is caused by *Leishmania donovani* and is transmitted by the bite of an infected female sand fly, *Phlebotomus argentipes*. The clinical features include progressive fever, weight loss, splenomegaly, hepatomegaly, hypergammaglobulinemia, and pancytopenia (Jeronimo et al. 2006). The disease is fatal in > 95% of untreated cases (Desjeux 1996), and with proper treatment, the case fatality rate will remain below 3%. This increased risk of death is often associated with jaundice, wasting, severe anemia, and HIV co-infection (Collin et al. 2004; Rey et al. 2005). The highest incidence countries are Sudan, South Sudan, Ethiopia, Somalia, India, and Brazil, and the highest burden of VL is currently in East Africa (WHO, Weekly Epidemiological Record 2017).

Nepal is topographically divided into three regions, namely, Terai (up to 600 m elevation), Hilly (600–3600 m elevation), and Himalayan (above 3600 m elevation). In the past, VL was endemic in the Terai region of Nepal, particularly in the 12 districts of the southeast region bordering the Bihar state of India (Fig. 1). These districts have been designated as leishmaniasis elimination program districts by the

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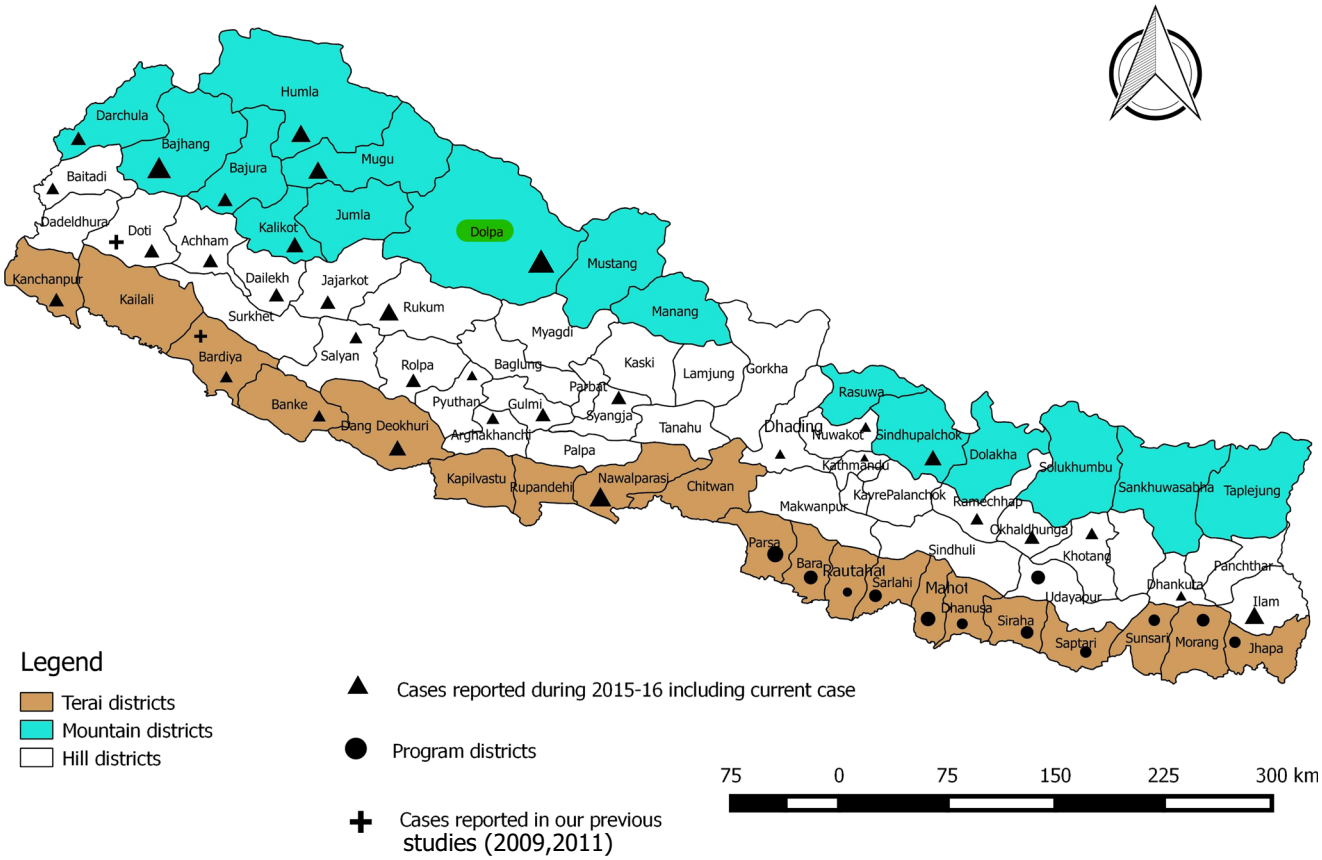

Fig. 1 District map of Nepal showing three ecological zones, 75 districts with programmed, previous case including the current case reported for VL

government of Nepal; however, almost 14% of the reported cases in 2013 were from other districts, termed as "non-program districts." Indian subcontinent countries, including Nepal, have committed to the elimination of VL as a public health problem by 2020 (National Strategic Guideline on Kala-azar Elimination Program in Nepal 2014). The lack of a VL public health infrastructure in non-program districts could pose a challenge in meeting the 2020 goal, necessitating a revalidation of the elimination methods of the program.

Background

In the years 2015 and 2016, a total of 168 VL cases were reported in Nepal, and of those 14% were from non-program districts (DoHS, Annual report 2015/16). Here, we report a case from the Himalayan region of Nepal; specifically, a 10-year-old boy who at the time of disease symptoms was studying at Lawan-3 in Dolpa (Fig. 1). The Dolpa district is the largest district of Nepal, with an area of approximately 8000 km^2, within the Himalayan region bordering Tibet (Statistical year book of Nepal 2013). Lawan village lies at approximately 5000 m elevation. The patient, weighing 23 kg, was initially admitted to the Dolpa District Hospital for diagnosis and management of fever. In July 2015, he was then referred to Kanti Children Hospital (KCH), Kathmandu, which is the only governmental referral hospital for children in the country. Demographic information such as name, gender, age, weight, address, and laboratory investigations were retrieved from the medical records. The patient had no history of travel to India or VL endemic regions of Nepal before the onset of fever. The patient suffered from fever and headache for 3 months. After a month of illness, the patient developed abdominal distension, which was gradually progressive when he visited KCH. The patient complained of a headache and had a cough since the first month of symptoms. He was found to have anemia, splenomegaly, hepatomegaly, and a lower respiratory tract infection.

Material and methods

Routine hematological tests were performed. Serological analysis was done using the rK39 rapid test (Insure; Inbios, Seattle, WA). The blood was used for molecular analysis, after spotting on filter paper and dried. The bone marrow was aspirated by a sternal puncture and used for smear preparation on a glass slide. The sample was fixed with methanol, stained with Giemsa solution, and observed under a microscope.

Polymerase chain reaction (PCR) was performed for species identification. DNA was extracted from Giemsa-stained slides of previously confirmed *L. donovani* culture isolates for

use as a positive control and from the patient's blood sample collected on filter paper, both using a DNeasy Blood and Tissue kit (QIAGEN, Valencia, CA) per the manufacturer's instruction. The final elutions were 50 μl from both the control and patient sample (Pandey et al. 2010). Nested PCR was performed using two sets of primers specific for the variable region of *Leishmania* kinetoplast minicircles, with minor modifications (primary PCR primers: CSB2XF – C/GA/GTA/GCAGAAAC/TCCCGTTCA and CSB1XR – ATTTTTCG/CGA/TTTT/CGCAGAACG; nested PCR primers: 13Z – ACTGGGGGTTGGTGTAAAATAG and LiR – TCGCAGAACGCCCCT) (Noyes et al. 1998). The conditions for the first round of PCR were 94 °C for 2 min, followed by 40 cycles of 94 °C for 30 s, 54 °C for 1 min, and 72 °C for 1.5 min. Diluted first-round PCR products were used as a template for 40 cycles of secondary PCR with nested primers, yielding a 720-bp product. Secondary PCR conditions were 94 °C for 2 min, followed by 94 °C for 30 s, 56 °C for 1 min, and extension at 72 °C for 40 s.

Results and discussion

Hematological findings showed that the patient had chronic anemia (hemoglobin level < 6.1 g/dl) and leucopenia (white blood cell count = 1500/mm^3) but normal platelets (130,000/mm^3). The rK39 test was positive for *Leishmania*. Microscopic examination of the bone marrow aspirate showed Leishman-Donovan (LD) bodies, indicating the presence of the parasite. PCR products from both the patient sample and the *L. donovani*-positive control confirmed *L. donovani* infection (Fig. 2). The patient was treated with liposomal amphotericin B (5 mg/kg). The patient improved, with decreased spleen size on discharge and normal hematological

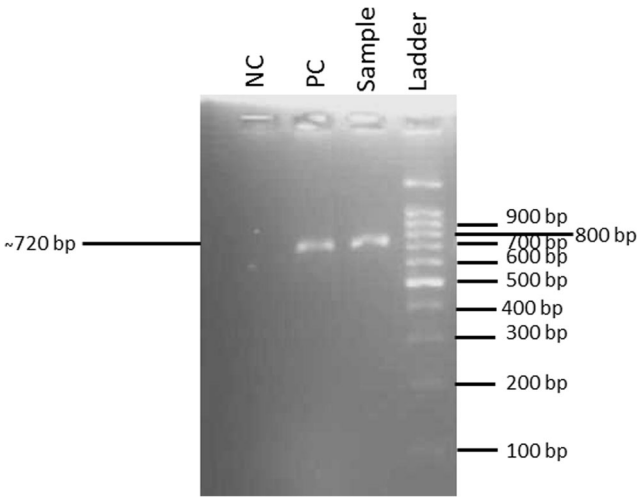

Fig. 2 Agarose gel electrophoresis of the PCR-amplified DNA fragments. The marker indicates a 100-bp DNA ladder. The sample referred to the sample collected from the patient. PC, positive control from DNA of *L. donovani* culture isolate; NC, negative control without DNA

parameters. Follow-up assessment was done at 12 months after treatment, at which time the patient was in good condition, and examination revealed no evidence of relapse.

This is the first report of VL from a non-endemic Himalayan region of Nepal, at an elevation of 5000 m above sea level. The patient had not traveled to any region having endemic or sporadic VL. We reported in 2009 VL from the non-program Bardiya district in the Terai southern plains region of Nepal (Pandey et al. 2009). Our group also reported in 2011 the first VL cases in the hilly region (Doti district) (Pandey et al. 2011). In our previous study, we found autochthonous VL cases mostly from non-endemic hilly regions of Nepal (Pun et al. 2013). Despite being considered a disease occurring at low elevations, as the disease is prevalent below 600 m above sea level, in recent years, VL has been observed to have expanded towards higher elevations as indicated by several case reports from hilly regions (Pandey et al. 2009; Pandey et al. 2011; Pun et al. 2013; Ostyn et al. 2015). Similarly, VL has also been reported from higher elevations, 3000 m above sea level, in neighboring India (Mahajan et al. 2004). This report provides an extreme example of such phenomena, as the patient was living at 5000 m elevation, with no travel history.

Dolpa district lies at an elevation from 1525 to 7625 m with no road links to neighboring districts and accessible only through air transport. The only one airport is situated at an elevation of 2500 m, and the flights operate to and from Nepalgunj (in Terai region). People living high altitudes like Lawan village (5000 m) have limited mobility due to no road access, and it is even less likely for kids (age of the current case). Despite this, we cannot totally exclude the meager possibility of being infected at relatively lower altitudes in Dolpa as we have no evidence to rule out. However, the data on VL cases reported in 2015–2016 (DoHS, Annual report 2015/16) from higher altitudes than previous years clearly indicate that VL is expanding towards higher altitude (Fig. 1). Therefore, based on the last few years of VL trend coupled with no history of blood transfusion in the patient and the living style (less mobility), it is not unlikely to have local transmission at this altitudes; nevertheless, it warrants for further entomological investigations and active surveys to establish. Alternatively, the sand fly vector and parasite are progressing to higher elevations; a scenario that has not been investigated by studies of vector prevalence. Published studies regarding the hilly regions of Nepal have reported that sand flies are present up to 1500 m elevation (Ostyn et al. 2015). Similarly, sand fly studies performed in Ethiopia have found the vectors at elevations of up to 2300 m (Yared et al. 2017). Although several leishmaniasis cases around the world have been reported among patients residing in higher elevations with no prior travel history to endemic or reported VL case regions, extensive studies in relation to the distribution of the sand fly remain a gap in our knowledge. In the present study,

we did not perform surveys of sand fly prevalence and active case surveillance to access infection rates in the community. Our findings underscore the need for entomological surveys, as well as case-based surveillance in higher elevation areas with collaboration with Department of Health Services, Government of Nepal, to inform both public health policy and VL elimination goals.

Conclusion

Despite the significant progress made towards VL elimination in Nepal, the emergence of cases in non-programmed areas likely represents a hindrance for sustained elimination and eventual eradication of the disease by 2020, as targeted by the government. This and recent studies suggest that VL is progressively appearing at higher altitudes. It is therefore our recommendation to strengthen surveillance with the inclusion of higher elevations, as well as non-program regions of Nepal.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study was approved by the Ethical Review Board of Nepal Health Research Council. The blood samples were collected after obtaining informed consent from the patient's parent and the concerned hospital.

References

Alvar J, Ve'lez ID, Bern C, Herrero M, Desjeux P, Cano J, Jannin J, den Boer M, The WHO Leishmaniasis Control Team (2012) Leishmaniasis worldwide and global estimates of its incidence. PLoS ONE 7(5):e35671. https://doi.org/10.1371/journal.pone.0035671

Collin S, Davidson R, Ritmeijer K, Keus K, Melaku Y, Kipngetich S, Davies C (2004) Conflict and kala-azar: determinants of adverse outcomes of kala-azar among patients in southern Sudan. Clin Infect Dis 38:612–619

Department of Health Services (DoHS), Ministry of Health and Population, Government of Nepal. DOHS Annual Report, 2015/2016; Kathmandu, Nepal: 2017

Desjeux P (1996) Leishmaniasis. Public health aspects and control. Clin Dermatol 14:417–423

Hotez PJ, Molyneux DH, Fenwick A, Ottesen E, Sachs SE, Sachs JD (2006) Incorporating a rapid-impact package for neglected tropical diseases with programs for HIV/AIDS, tuberculosis, and malaria. PLoS Med 3(5):e102. https://doi.org/10.1371/journal.pmed.0030102

Jeronimo SMB, de Queiroz Sousa A, Pearson RD (2006) Leishmaniasis. In: Guerrant RL, Walker DH, Weller PF (eds) Tropical infectious diseases: principles, pathogens and practice. Churchill Livingstone Elsevier, Edinburgh, pp 1095–1113

Mahajan SK, Machhan P, Kanga A, Thakur S, Sharma A, Prasher BS, Pal LS (2004) Kala-azar at high altitude. J Commun Dis 36(2):117–120

National Strategic Guideline on Kala-azar Elimination Program in Nepal (2014) Epidemiology and Disease Control Division, Department of Health Services, Ministry of Health and Population, Government of Nepal. pp 1–2

Noyes HA, Reyburn H, Bailey JW, Smith D (1998) Nested-PCR-based Schizodeme method for identifying leishmania kinetoplast minicircle classes directly from clinical samples and its application to the study of the epidemiology of Leishmania tropica in Pakistan. J Clin Microbiol 36(10):2877–2881

Ostyn B, Uranw S, Bhattarai NR, Das ML, Rai K, Tersago K, Pokhrel Y, Durnez L, Marasini B, Van der Auwera G, Dujardin JC, Coosemans M, Argaw D, Boelaert M, Rijal S (2015) Transmission of Leishmania donovani in the hills of Eastern Nepal, an outbreak investigation in Okhaldhunga and Bhojpur districts. PLoS Negl Trop Dis 9(8):e0003966

Pandey BD, Pandey K, Kaneko O, Yanagi T, Hirayama K (2009) Short report: relapse of visceral leishmaniasis after miltefosine treatment in a Nepalese patient. Am J Trop Med Hyg 80(4):580–582

Pandey K, Pandey BD, Mallik AK, Kaneko O, Uemura H, Kanbara H, Yanagi T, Hirayama K (2010) Diagnosis of visceral leishmaniasis by polymerase chain reaction of DNA extracted from Giemsa's solution-stained slides. Parasitol Res 107:727–730

Pandey BD, Pun SB, Kaneko O, Pandey K, Hirayama K (2011) Case report: expansion of visceral leishmaniasis to the western hilly parts of Nepal. Am J Trop Med Hyg 84(1):107–108

Pun SB, Pandey K, Shah R (2013) A series of case reports of autochthonous visceral leishmaniasis, mostly in non-endemic hilly areas of Nepal. Am J Trop Med Hyg 88(2):227–229

Rey LC, Martins CV, Ribeiro HB, Lima AA (2005) American visceral leishmaniasis (kala-azar) in hospitalized children from an endemic area. J Pediatr 81:73–78

Statistical year book of Nepal (2013) Central bureau of statistics, National Planning commission Secretariat, Government of Nepal

WHO Leishmaniasis Sheet (2017) Available from http://www.who.int/mediacentre/factsheets /fs375/en/. Accessed on 29 Aug 2017

WHO, Weekly Epidemiological Record, No 38, 2017, 92:557–772

Yared S, Gebresilassie A, Akililu E, Deribe K, Balkew M, Warburg A, Balke M, Warburge A, Hailu A, Gebre-Michael T (2017) Diversity and altitudinal distribution of phlebotomine sand flies (Diptera: Psychodidae) in visceral leishmaniasis endemic areas of northwest Ethiopia. Acta Trop 176:1–10