International Journal of Infectious Diseases 86 (2019) 171–174

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Case Report

Recent autochthonous cases of leishmaniasis in residents of the Republic of Dagestan, Russian Federation

Sergey Andreevich Nagorny[a], Larisa Alexandrovna Ermakova[a,b], Natalia Vladimirovna Golovchenko[a], Natalia Yurievna Pshenichnaya[c], Andrey Sergeevich Zhuravlev[d], Trentina Di Muccio[e], Luigi Gradoni[e,*]

[a] Rostov Scientific Research Institute of Microbiology and Parasitology, Rostov-on-Don, Russia
[b] Rostov State Medical University, Rostov-on-Don, Russia
[c] Central Research Institute of Epidemiology and National Medical Research Center of Phthisiopulmonology and Infectious Diseases, Moscow, Russia
[d] First Moscow State Medical University, Moscow, Russia
[e] Unit of Vector-Borne Diseases, Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy

ARTICLE INFO

Article history:
Received 12 April 2019
Received in revised form 5 July 2019
Accepted 5 July 2019
Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
Visceral leishmaniasis
Cutaneous leishmaniasis
HIV infection
Dagestan
Russian Federation

ABSTRACT

Eighty years after the last published record of human leishmaniasis from Dagestan, Russian Federation, we report two recent cases which were most probably acquired locally: one case of visceral leishmaniasis in a 2-year old child, and one cutaneous leishmaniasis case in a 39-year-old man co-infected with HIV, both resident in Dagestan.

Introduction

Leishmaniases are endemic in over 98 countries, with 0.3 million visceral leishmaniasis (VL) and one million cutaneous leishmaniasis (CL) cases, respectively, estimated to occur annually (Alvar et al., 2012). These two clinical forms are also found in temperate zones of the WHO European Region, from Portugal at west to Kazakhstan at east (Gradoni et al., 2017). Throughout this territory, *Leishmania infantum* is the only causative agent of VL, having dogs as the main reservoir host and several phlebotomine sand fly species as vectors. A recent review of VL in countries of the former Soviet Union highlighted southern Caucasus (i.e. Azerbaijan, Georgia and Armenia) as the territory most affected by disease resurgence over the past decade (Strelkova et al., 2016). As regards the Russian Federation, only two autochthonous cases from northern Caucasus were recorded in the past, one of which from Dagestan in 1938. Isolated VL cases were registered in Crimea (Baranets et al., 2017), whereas CL diagnosed in the Russian Federation is only regarded as an imported disease by health authorities.

We have recently diagnosed two cases of leishmaniasis in residents of Dagestan Republic, which were most probably acquired locally. The protozoan agent, unfortunately left untyped, could be attributable to *L. infantum* on clinical and epidemiological grounds.

Case 1

In June 2014, a 2-year-old girl from Makhachkala, Dagestan, was referred to the infectious and parasitic diseases clinic of Rostov-on-Don Research Institute of Microbiology and Parasitology (RNIIMP), Russia. According to her parents, the girl felt ill in January 2014, when she started to suffer from unexplained fever. In May 2014 parents sought medical assistance, as the child's condition had worsened resulting in weight loss, persisting fever, abdominal pain and enlargement, and profuse sweating. The child received symptomatic therapy at the Children's Hospital in Makhachkala and subsequently referred

* Corresponding author at: Unit of Vector-Borne Diseases, Department of Infectious Diseases, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy.
E-mail address: luigi.gradoni@iss.it (L. Gradoni).

https://doi.org/10.1016/j.ijid.2019.07.005

to the Rostov-on-Don Research Institute of Oncology, where cancer or blood malignancies were excluded. The child was transferred to the pediatric infectious diseases ward of the city hospital, where anemia, thrombocytopenia, leucopenia and hepatosplenomegaly were recorded. Due to suspicion of a parasitic infection, the patient was sent for consultation to RNIIMP.

On the day of examination, remarkable blood findings were hemoglobin 98 g/L and leukocytes 3.8×10^9/L. Formol-gel test, an aspecific *Leishmania* serology assay (World Health Organization, 1996) was strongly positive. For the above clinical and laboratory features, a sternal bone-marrow aspiration was performed and Romanowsky-Giemsa stained smears revealed the presence of *Leishmania* amastigotes (Figure 1A), confirming that the child had VL. For the child's age and the incubation duration, it was concluded that the infection was acquired in Makhachkala.

After VL diagnosis, the child was sent back to the Makhachkala Children's Hospital for treatment. Because first-line drugs for treatment of VL (i.e. meglumine antimoniate or liposomal amphotericin B) are not registered in the Russian Federation, the patient had to be treated with intravenous amphotericin B deoxycholate, which caused adverse effects (nausea, vomiting and weakness) and had to be interrupted. With the help of relatives, the child was transferred to a hospital in the Republic of Azerbaijan for further treatment; there, she was successfully treated with liposomal amphotericin B administered at the dose of 5 mg/kg/day for 10 days. As of today, no VL relapses were recorded.

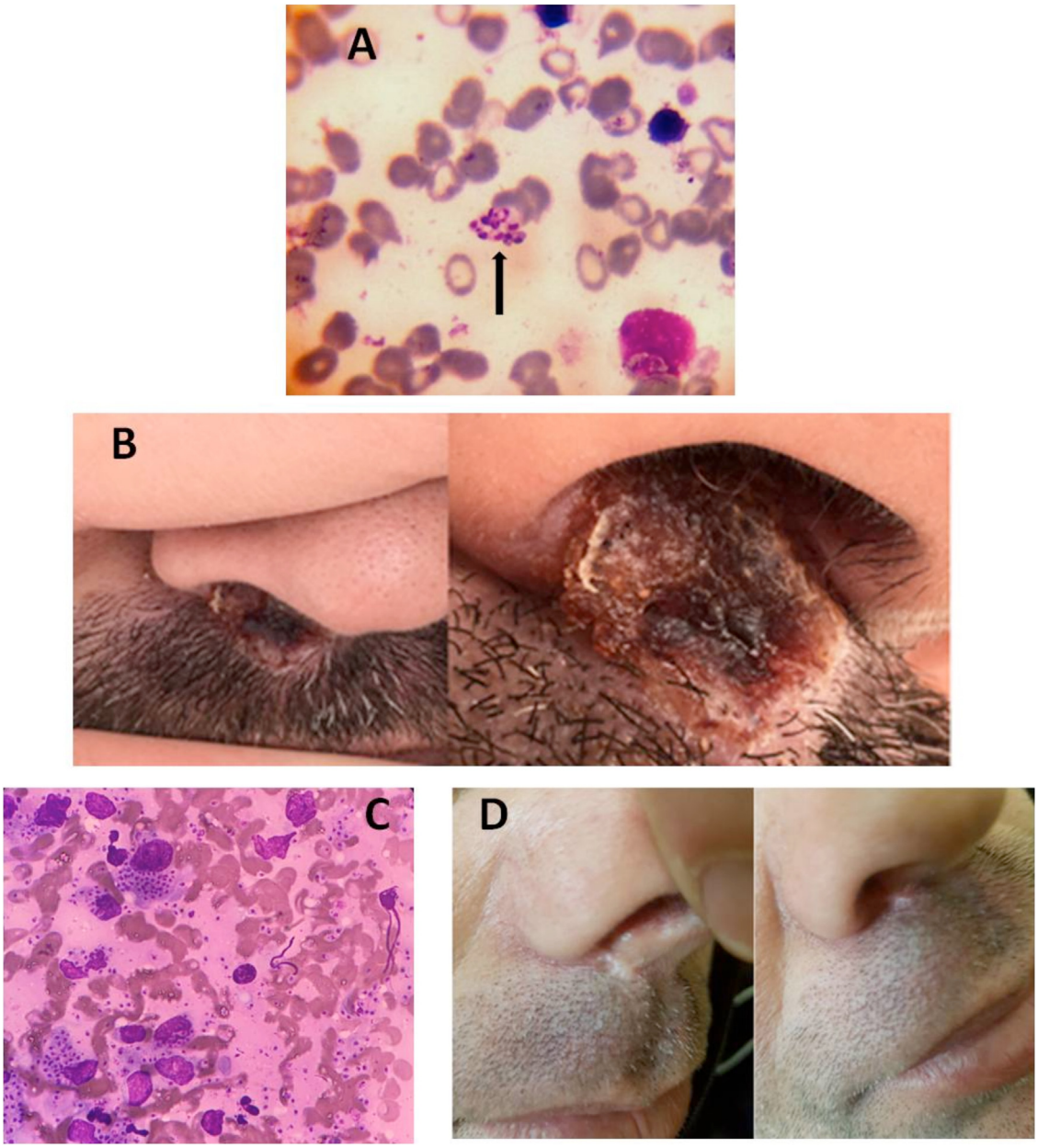

Figure 1. *Leishmania* diagnosis in Case 1 and 2, and clinical features of Case 2.
(A) Cluster of *Leishmania* amastigotes within cytoplasm residues from a broken infected macrophage (arrow), in a bone-marrow smear from Case 1, a 2-years old child. Romanovsky-Giemsa stain; (B) Ulcer on the border between the upper lip and right nostril of Case 2, an adult male co-infected with HIV and *Leishmania*; (C) Numerous *Leishmania* amastigotes in the smear from the skin ulcer of Case 2. Romanovsky-Giemsa stain; (D) Scar at the site of the ulcer after Case 2 treatment.

Case 2

In January 2018, a 39-years-old man from a rural area of Dagestan was referred to the RNIIMP clinic from the Rostov-on-Don Research Institute of Oncology. The patient felt ill in November 2017, when a papule appeared at the border of the upper lip and right nostril, which after several days turned into an ulcer. He was treated by a dermatologist at his place of residence, without results. He was then referred to a regional oncologist who suspected a basal-cell carcinoma and excised the ulcer. After surgery, the patient's condition worsened, with fluctuating fever and sharp pain in the intervention area; an ulcerative lesion developed again in the postoperative suture and gradually increased in size. The patient was referred to the Rostov-on-Don Research Institute of Oncology with suspicion of malignant skin formation. The ulcer (2.1×1.8 cm) was evident at the border between the upper lip and right nostril (Figure 1B). Surrounding skin was infiltrated, hyperemic, and covered with small tubercles. Occipital, posterior cervical and axillary lymph nodes were enlarged. Material for parasitological examination was taken from the infiltrate around the wound, from which slide smears were made and stained with Romanovsky-Giemsa's stain. At microscopic examination, numerous *Leishmania* amastigotes were detected (Figure 1C) and hence CL was diagnosed. At the epidemiological investigation, the patient categorically denied having visited neighboring countries known to be endemic for CL caused by *L. major* and/or *L. tropica*. Molecular typing of the agent was attempted from stained slide smear material, after abundant washing in ethanol and removal. DNA was extracted by standard proteinase K protocol (Khatri et al., 2009) and analyzed by nested-PCR targeting the SSU rDNA, and by Real-Time PCR targeting kDNA minicircles (Van Eys et al., 1992; Mary et al., 2004). Integrity of human b-globin gene DNA was also assessed (Pizzuto et al., 2001). Unfortunately, neither human nor *Leishmania* DNA were amplified, suggesting probable inhibition caused by residual traces of the dye.

Clinical laboratory investigations revealed lymphopenia and monocytosis. Formol-gel test for *Leishmania* serology was negative, however anti-HIV 1 + 2 ELISA tested positive. The Dagestan Center for HIV/AIDS confirmed that the patient was HIV infected since 2010. Indicators of the immune status at the time of the investigation were: absolute T-lymphocytes (CD45+/CD3+): 498×10^6/L; T-helpers (CD45+/CD3+/CD4+): 76×0^6/L; cytotoxic T-lymphocytes (CD45+/CD3+/CD8+): 422×10^6/L; double positive T-lymphocytes (CD3+/CD4+/CD8+): 0.34×10^6/L; immunoregulatory index CD4/CD8: 0.18. HIV load was 4,473,014 copies/mL. Hence, definitive diagnosis was CL in an individual co-infected with HIV, stage 4B. Considering the high risk for a generalized leishmaniasis, the patient was treated intravenously with conventional amphotericin B, 50000 units per day every day for 7 days, then once a week for 30 weeks. The ulcer was treated locally with 1% clotrimazole ointment twice a day. The patient has been prescribed antiretroviral therapy (LPV/RTV + ZDV+3TC). Treatments were well tolerated without significant side effects. After 42 days from the start of therapy with amphotericin B and antiretroviral therapy, the patient's condition improved and the ulcer was almost completely healed (Figure 1D). Positive dynamics of the immune status indicators were noted: CD4+ increased to 233×10^6/L, and HIV RNA decreased to 1,416 copies/ml. In view of the regression of the CL lesion, amphotericin B treatment was interrupted. Currently, the patient is receiving ART, clinical conditions are satisfactory, and adherence to HIV therapy is high.

Discussion

According to official data, only imported leishmaniasis is diagnosed in the Russian Federation. A 2015 survey carried out on previous 4 years (Federal Service for Surveillance on Consumer Rights Protection and Welfare, 2015) concluded that the disease was acquired from eleven countries, i.e. Azerbaijan, Israel, Indonesia (actually a country non-endemic for leishmaniasis), Iran, Spain, Mexico, Syria, Tajikistan, Turkmenistan, Turkey and Uzbekistan. The highest number of VL cases was imported from Azerbaijan.

Based on Case-1 history, endemic *Leishmania* transmission in Dagestan seems to be undisputable. Occurrence of autochthonous VL in the country should not be surprising, as southern and southwestern parts of Dagestan border with VL-endemic Azerbaijan and Georgia (see https://en.wikipedia.org/wiki/Dagestan for a dedicated map). Two competent *L. infantum* vectors (*Phlebotomus kandelakii* and *P. neglectus*) had been recorded in the 1930s from the Caspian Sea coastline and foothills of Dagestan (Strelkova et al., 2016), suggesting natural conditions for local leishmaniasis transmission. In severely immunosuppressed individuals, the multiplication of *L. infantum* in tissues can occur atypically, most often involving skin and/or mucous membranes as a result of secondary spread, thereby causing CL or mucosal leishmaniasis (Alvar et al., 2008). Case 2 may have suffered from this condition, however it cannot be excluded that the lesion developed in the site of an infective sand fly bite.

Sporadic locally-acquired cases in territories considered free from leishmaniasis may remain unnotified to the public health system, with consequent lack of appropriate surveillance responses (e.g. *Leishmania* screening in dog populations). Furthermore, these cases may lead to serious problems in timely diagnosis and treatment, as physicians usually have no awareness of the condition and knowledge of methods for laboratory confirmation (Pshenichnaya et al., 2017). It is therefore important to provide public health and medical specialists with information about the current epidemiological situation and with correct approaches to diagnose and treat leishmaniasis in a timely manner.

Conflict of interest statement

The authors declare no conflict of interest

Funding source

This study did not receive specific funds other than those routinely provided by public hospitals for patient care.

Ethical statement

Management of patients followed current clinical guidelines of the Russian Federation.

References

Alvar J, Aparicio P, Aseffa A, Den Boer M, Cañavate C, Dedet JP, et al. The relationship between leishmaniasis and AIDS: the second 10 years. Clin Microbiol Rev 2008;21:334–59.

Alvar J, Vélez ID, Bern C, Herrero M, Desjeux P, Cano J, et al. Leishmaniasis worldwide and global estimates of its incidence. PLoS One 2012;7:e35671.

Baranets MS, Ermak TN, Ponirovsky EN. Clinical and epidemiological features of visceral leishmaniasis in the Republic of Crimea. Ther Arch (Mosk) 2017;89:100–4 [in Russian].

Federal Service for Surveillance on Consumer Rights Protection and Welfare, the Russian Federation. [About the situation of leishmaniasis in the Russian Federation]. Letter of 4th April 2015, no. 01/2160-15-27. http://docs.cntd.ru/document/420268897. [in Russian].

Gradoni L, López-Vélez R, Mourad M. Manual on case management and surveillance of the leishmaniases in the WHO European Region. Copenhagen: World Health Organization Regional Office for Europe; 2017.

Khatri ML, Di Muccio T, Gramiccia M. Cutaneous leishmaniasis in North-Western Yemen: a clinicoepidemiologic study and Leishmania species identification by polymerase chain reaction-restriction fragment length polymorphism analysis. J Am Acad Dermatol 2009;61:e15–21.

Mary C, Faraut F, Lascombe L, Dumon H. Quantification of *Leishmania infantum* DNA by a real-time PCR assay with high sensitivity. J Clin Microbiol 2004;42:5249–55.

Pizzuto M, Piazza M, Senese D, Scalamogna C, Calattini S, Corsico L, et al. Role of PCR in diagnosis and prognosis of visceral leishmaniasis in patients coinfected with human immunodeficiency virus type 1. J Clin Microbiol 2001;39:357–61.

Pshenichnaya NY, Paul M, Erovichenkov A. Eastern Europe. In: Petersen E, Chen LH, Schlagenhauf-Lawlor P, editors. Infectious diseases: a geographic guide. Oxford: John Wiley & Sons; 2017. p. 235–56.

Strelkova MV, Ponirovsky EN, Morozov EN, Zhirenkina EN, Razakov SA, Kovalenko DA, et al. A narrative review of visceral leishmaniasis in Armenia, Azerbaijan, Georgia, Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan, Uzbekistan, the Crimean Peninsula and Southern Russia. Parasites Vectors 2016;8:330.

Van Eys GJ, Schoone GJ, Kroon NC, Ebeling SB. Sequence analysis of small subunit ribosomal RNA genes and its use for detection and identification of Leishmania parasites. Mol Biochem Parasitol 1992;51:133–42.

World Health Organization. Manual of visceral leishmaniasis control Geneva: WHO/LEISH/96.40. 1996.