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Retrospective analysis of visceral leishmaniasis relapses in immunocompetent patients who received treatment with 20mg/kg liposomal amphotericin B in Bihar, India

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Background: India reportedly has 70% of the worldwide burden of Visceral Leishmaniasis(VL), with Bihar having 90% of reported cases. With the support of the RMRI, MSF has implemented a VL treatment project in Vaishali district, endemic for Leishmania donovani, using total dose 20mg/Kg liposomal amphotericin-B(L-AmB) as the first-line drug.

Methods: Intravenous L-AmB(Ambisome) has been administered in four doses of 5mg/kg/doses to a total dose of 20mg/kg to all patients identified as primary VL over a period of 5 to 10 days depending on the severity of disease. All patients are routinely given comprehensive health education regarding VL and instructed to return immediately if any recurrence of symptoms occurred. The Excel based database of the project was analysed over a 4-year period from September 2007-December 2011. All immunocompetent patients readmitted with biopsy confirmed VL who had been previously treated for primary VL within the programme were identified, described and compared to the parameters of overall admissions of immunocompetent patients with primary VL within the same period.

Results: 6435 immunocompetent primary VL patients were treated during the analysis period with 20mg/Kg LAmb. 80 of these patients re-presented with parasite confirmed relapses. This constituted a minimum of 1.2% of the overall treated, however passive follow up may underestimate the real number.The parameters of the relapse cases are compared to the baseline data from the overall admissions in Table 1. Male sex and shorter time from symptoms to diagnosis were associated with risk of VL relapse.The average (SD) length of time following completion of initial treatment to parasite confirmation was 385 (272) days (range:104 -1626), with 59% occurring between 6 and 12 months (Table 2).**Conclusion:** To our knowledge this is the only study of relapses in immunocompetent primary VL patients treated with L-AmB. Although passive follow up limits interpretation, only male sex and short treatment delay seem to be associated with risk of VL relapse. The majority of relapses occurring between 6-12 months post treatment is of interest and may suggest that a longer period of follow up could be appropriate for patients treated with L-Amb in light of WHO recommendations for 10mg single dose therapy.

Table 1
Comparison of relapse case parameters to baseline admissions

	Relapse		Baseline				
Risk Factor	N	%	N	%	RR	95% CI	P-Value
Age							
<5	7	8.8	428	6.7	1.34	(0.60-2.98)	0.47
5-15	33	41.3	2632	41.4	1.03	(0.65-1.63)	0.89
>15	40	50	3295	51.8	1		
Gendet							
M	59	73.8	3531	55.6	2.22	(1.36-3.66)	**0.001**
F	21	26.3	2824	44.4	1		
Time from symptoms onset to diagnosis							
≤2 weeks	24	30	918	14.4	1		
2-4 weeks	34	42.5	2754	43.3	0.48	(0.2-0.80)	0.004
4-8 weeks	15	18.8	1632	25.7	0.36	(0.19-0.68)	0.001
8-12 weeks	4	5.0	669	10.5	0.25	(0.08-0.67)	0.003
>12 weeks	3	3.8	382	6.0	0.31	(0.09-1.01)	0.038
Hb (g/dl)							
<6	10	13.7	840	13.2	0.98	(0.49-1.95)	0.96
6-8	22	30.1	2132	33.5	0.85	(0.31-1.42)	0.54
>8	41	56.2	3383	53.2	1		
Spleen Size							
<3 cm	13	17.8	995	15.7	1		
3-6 cm	43	58.9	3279	51.6	1.004	(0.54-1.86)	0.99
<6 cm	17	23.3	2078	32.7	0.63	(0.31-1.29)	0.20
Nutritional Status							
Severe Acute Malnutrition	12	19.4	956	18.9	1.13	(0.59-2.18)	0.72
Moderate Acute Malnutrition	17	27.4	1138	22.5	1.34	(0.75-2.40)	0.32
Normal Nutritional Status	33	53.2	2971	58.7	1		

	Age at primary diagnosis			
	<5	5-15	>15	Total (%)
Time from completion of treatment to diagnosis of relapse				
<3 months	0	0	0	0 (0)
3-6 months	0	3	4	7 (8.8)
6-9 months	3	14	8	25 (31.3)
9-12 months	1	7	14	22 (27.5)
12-18 months	1	7	5	13 (16.3)
>18 months	2	2	9	13 (16.3)
Total	7	33	40	80

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Invasion kinetics of human endothelial cells by *Toxoplasma gondii* RH and ME49 strains

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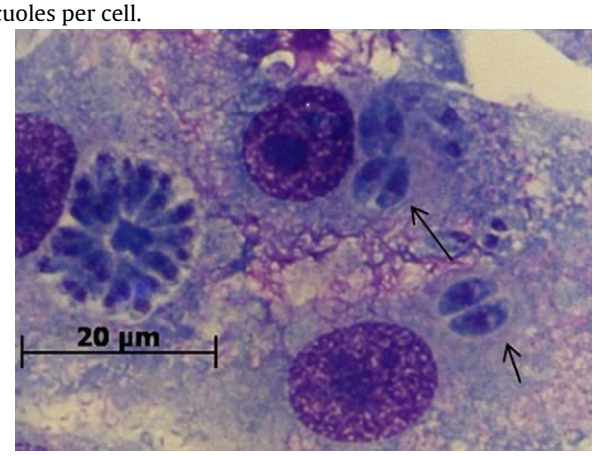
Background: *Toxoplasma gondii* may cause congenital infection in the developing foetus. Invasion of endothelial cells lining the placental blood vessels is supposed to be the main vertical transmission route. Genotypic analysis of *T. gondii* isolates identified a population structure of 11 haplogroups, from which three clonal lineages, type I, II, and III are well known. Although, type II strains are more prevalent in Europe and North America, type I strains are over-represented in congenital toxoplasmosis; also, type I strains are highly virulent to mice and traverse epithelial barriers more effectively than type II. The aim of this study was to compare the invasion kinetics of *T. gondii* RH and ME49 strains in human microvascular endothelial cells(HMEC-1) and umbilical vein endothelial cells (HUVECs).

Methods: RH and ME49 *Toxoplasma gondii* strains were expanded in Balb/c and C57BL6-RAG2 -/- mice, respecitvely. After one replication cycle in VERO cell cultures (figure) tachyzoites were seeded at 10:1 parasite:cell ratio in 24-well plates containing slides with monolayers of either HMEC-1 or HUVECs, at 100,000/well and incubated for 30 min to 4h. The slides were fixed and stained with Wright to count percent of infected cells and number of parasitic

vacuoles per cell.



Toxoplasma gondii RH tachyzoites replicated in VERO cell cultures. A rosette of many tachyzoites is shown at left and several parasite pairs at right (arrows)

Results: In both cell types invasion with either strain increased along time; however, proportion of infected cells was lower for HUVECs than for HMEC-1. Also, the strains differed in invasion kinetics: ME49 parasites were faster than RH ones, regardless of cell type. Finally, both HMEC-1 and HUVECs showed higher number of parasitic vacuoles per cell when infected by ME49 tazhyzoites than by RH protozoan, i.e ≈30 vs ≈20 at 4 hours, respectively.

Conclusion: Results suggest that HMEC-1 cells are more susceptible to infection by *T. gondii* than HUVECs. This might be related to cell cycle-progress -which is usually badly regulated in cell lines- and *T. gondii* is more invasive during the G1-S phase. The unexpected observation that RH parasites are slower than ME49 ones might be related to their higher ability to survive out of the cell.

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Seroprevalence of *Trypanosoma cruzi* in immigrant women living in Basque Country, Spain

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Background: Chagas disease or American trypanosomiasis represents a public health problem all over the world, principally due to immigration. Detection of this infection in non endemic areas is essential to prevent congenital transmission, horizontal transmission and Chagas disease. This study aims to show the prevalence on infection due to *Trypanosoma cruzi* in women from endemic areas living in Basque Country, Spain.

Methods: Blood samples were collected from Latin American immigrant women. Overall, studied women were from 12 different countries and they are living in Basque Country between 1 and 7 years. The samples were screened by three rapid and conventional serological tests: indirect immunofluorescence assay (IFA) and two indirect ELISA, ORTHO (second generation) and DIA.PRO (third generation).

Results: Overall, 270 women were analyzed and 51 (18.8%) were seropositive. Using IFA, there were two more positive samples, but there were considered false positive. Most of the seropositive women were from Bolivia (90.2%), following Paraguay (5.9%) and Brazil (3.9%). Age range from seropositive women were slightly older than seronegative women (32 vs. 29, p>0.05). Prevalence in women with familial history of Chagas disease were higher than in women without any history (37.3% vs. 16.4%, p=0.001). Moreover, the prevalence was higher in women who had lived in adobe house than in brick house (16% vs. 3.6%, p=0.002).

Conclusion: This study tightens that screening programs are necessary to evaluate the prevalence of *Trypanosoma cruzi* in immigrant population in order to prevent blood transfusion transmission or vertical transmission. Indirect ELISA techniques are rapid and suitable to detect IgG antibodies in immigrant population.

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Recurrent diffuse cutaneous leishmaniasis

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Background: Little has been said about recurrent diffuse cutaneous leishmaniasis (RDCL) in mediterranean area. We came across two cases with multiple leishmaniasis, but not recurrent: one in immunocompetent subject and the other in a HIV positive person.

Aim. To present a case with RDCL imported from Iraq.

Methods: There were analyzed all aspects such as epidemiologic, clinic-diagnostic and therapeutic and was followed up for 18 months.

Results: Case presentation. A healthy male, 47 years-old, who after three years living in Mosul, Iraq develops two papulos elements in both elbows. After 7-10 days other elements developed, first on the left arm, left ear and in the right leg. Those elements became bigger in size and within three weeks they reached the maximal size. Treatment with cortizon, antymycotice locally, amoxiclav and ceftriaxon had no effect. He was sent to our clinic and was hospitalized. There were noticed 13 elements papulo-ulcero-granulomatosis from which only two were moisten. Microbiological researches: direct microscopy evidenced the existence of leishmania in 3 of lesions, from one moist element we isolated S.aureus. Direct examination and the culture for mold resulted negative. Hystopathologjical research noticed mononuclear infiltration and any giant cell and ruled out tumor pathology and discotic lupus. In other systems – no pathology signs and symptoms. Biological and immunological research was normal.HIV negative.The case was treated with glukantime 1.5 g/5ml x2/die for 28 days. After 6-7 day we noticed lesion diminution and patient discharged on the 28nd day improved about 80%. Fluconazol was prescribed for 20 days at home if not clean properly