

***Pneumocystis* species : parasites of the lung of mammals**



- Guinea pig, other Caviomorphs
- Wild rats
- Voles (*Apodemus sylvaticus*, *Microtus agrestis*)
- Squirrels



- Dog, domestic cat, red fox, ferret, fennec, grison, panda



- Rabbits (PcP at weaning)
- Hares (*Lepus europaeus*)



- Shrews (*Sorex araneus*, *Notiosorex crawfordi*)
- Mole (*Talpa europaea*)



- Lemurs
- Humans, apes, other Old World monkeys
- New World monkeys



- Pig - outbreaks of PcP in herds
- Horse - PcP in SCID foals
- Cow, goat, sheep, antilopes

Bats



***Pneumocystis* spp. from 3 bat species**

☒ Tropical Rainforest

Marsupials



***Pneumocystis* sp. from 1 species**

☒ Tropical Rainforest

Cetaceans

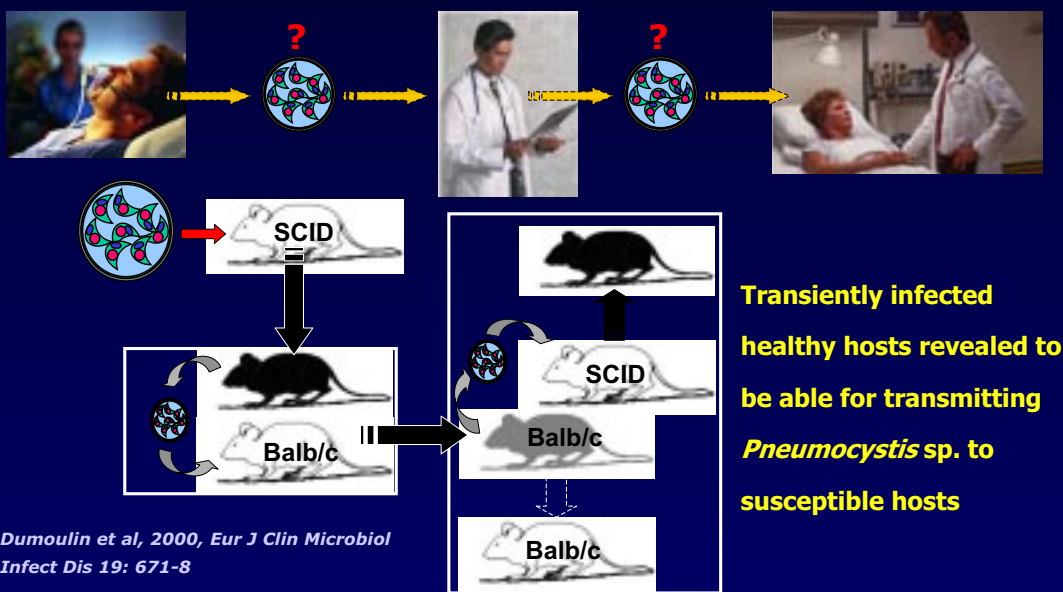


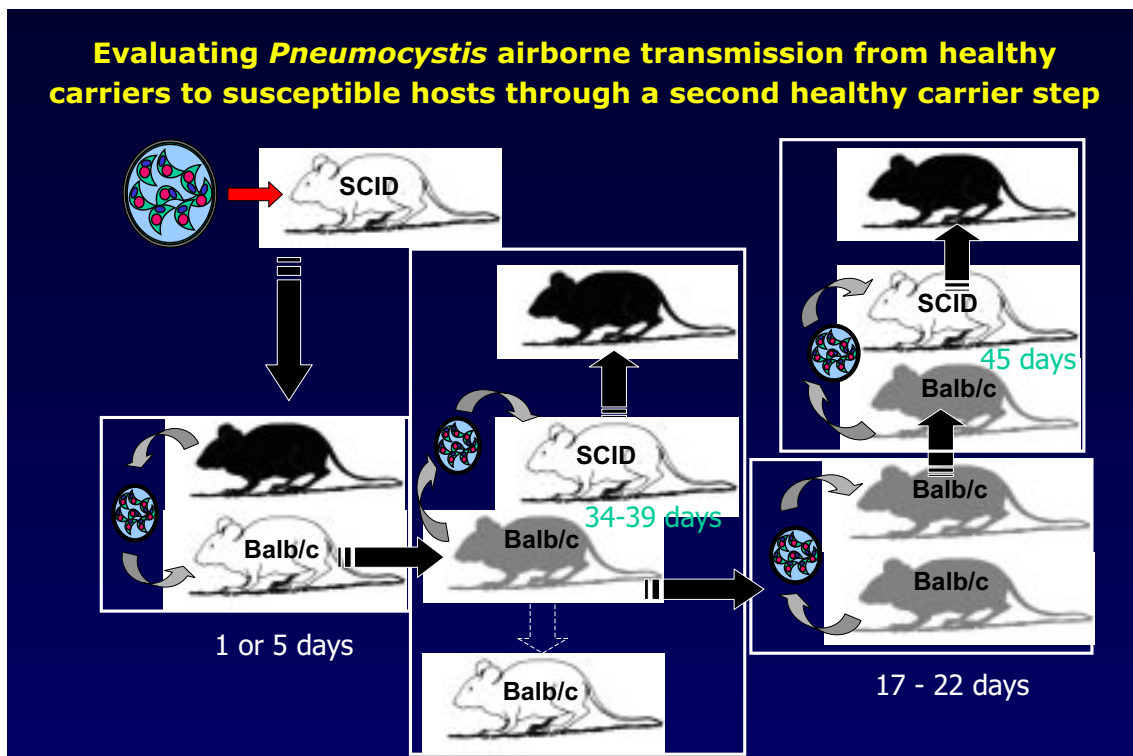
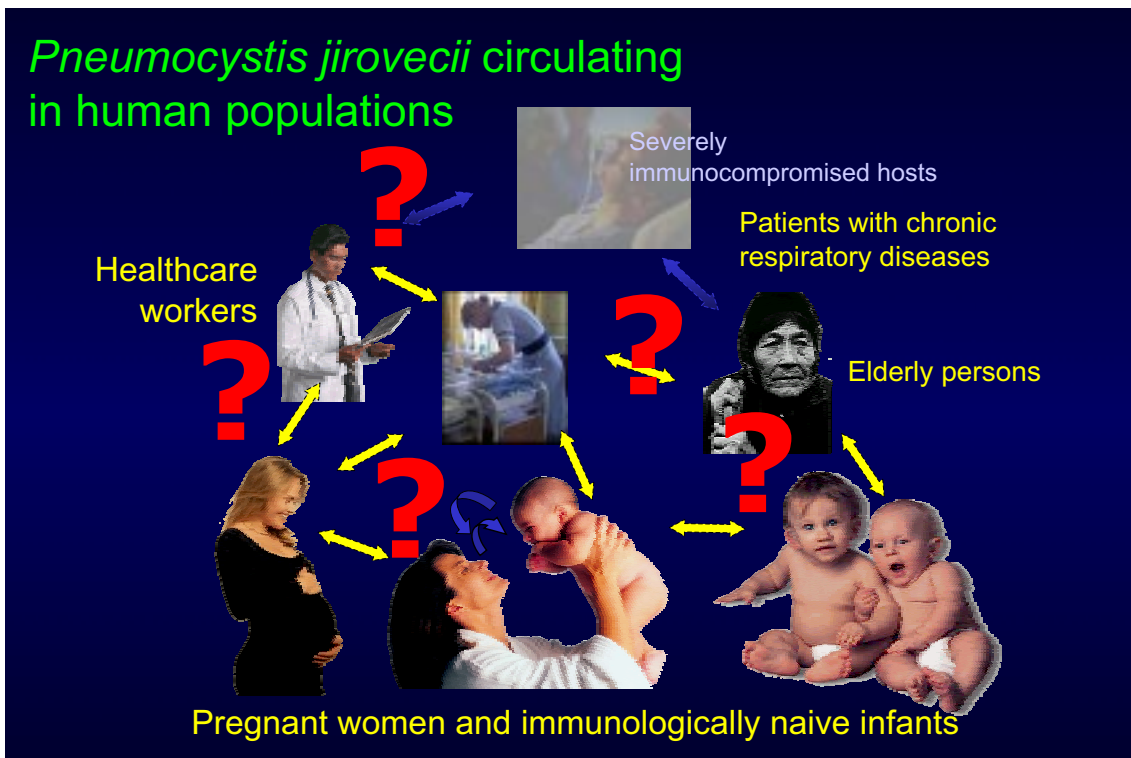
***Pneumocystis* sp. from 1 species (needs confirmation)**

☐ Marine biome



Evaluating *Pneumocystis* airborne transmissibility between hosts with PcP, carriers and susceptible hosts





What about healthy humans as *Pneumocystis* carriers ?

- *Pneumocystis* DNA was detected in healthcare contacts of PcP patients

Vargas et al, 2000, JCM 38: 1536-8; Miller et al, 2001, JCM 39: 3877-82

- No *Pneumocystis* carrier was detected in a Pneumology Unit (0/35), which is in charge of immunocompetent adults
- Carriers were detected in 8 % careworkers (13 / 164) from services with either numerous immunocompetent children or a high proportion of immunocompromised patients

Durand-Joly et al, 2003. J Euk Microbiol (in press)

- Carriers could play a role:
 - in the circulation of *P. jirovecii* in hospitals
 - of reservoir to *Pneumocystis* species

Dei-Cas, 2000, Med Mycol 38 (Suppl 1): 23-32

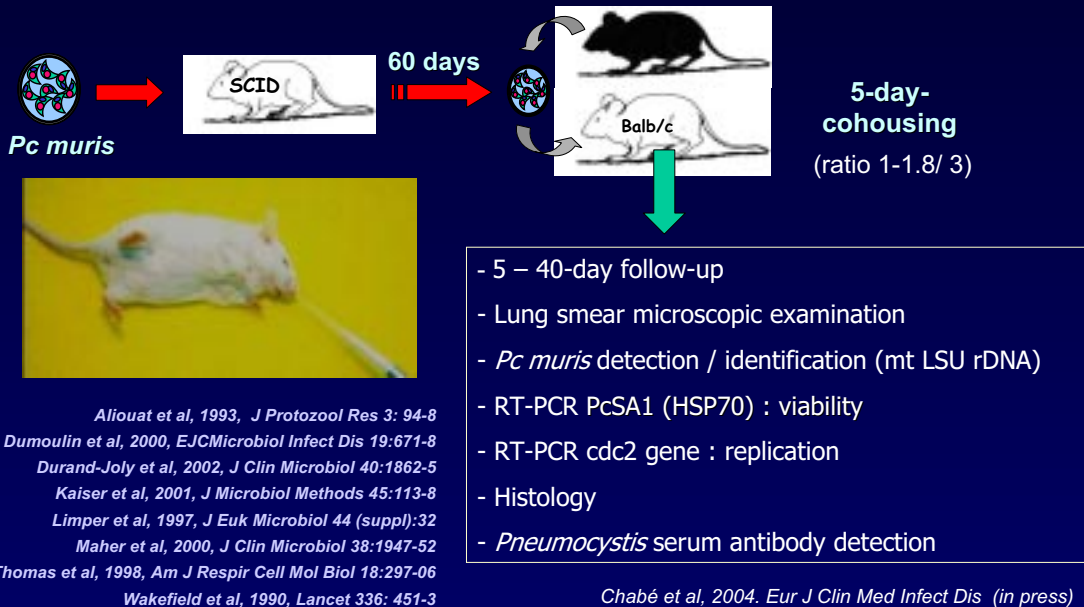
Durand-Joly et al, 2003, JEM (in press)

Gigliotti et al, 2003, Infect Immun 71:3852-6

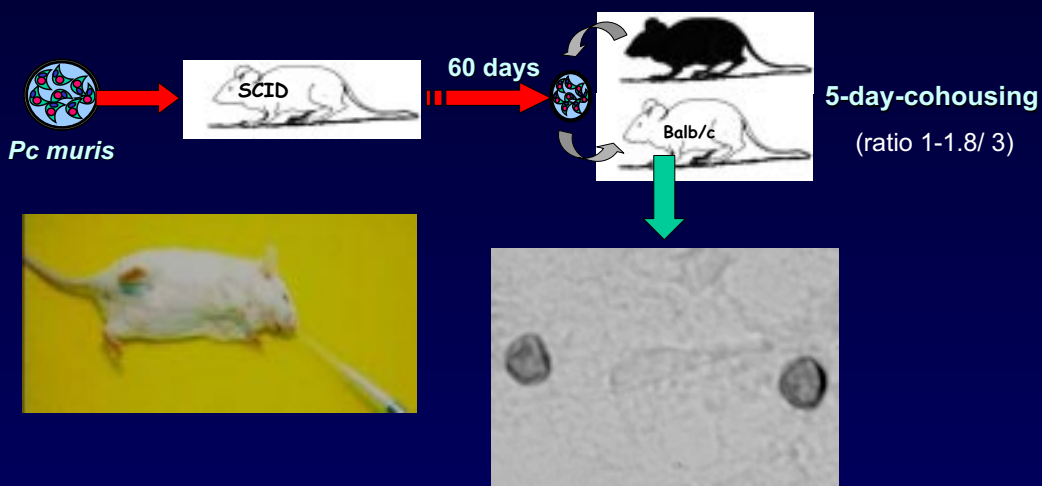
Pneumocystis in the lung of healthy hosts ...

- Exploring the behaviour of *Pneumocystis* organisms in healthy carrier lungs:
 - latency ?
 - active replication ?

Methodological Strategy



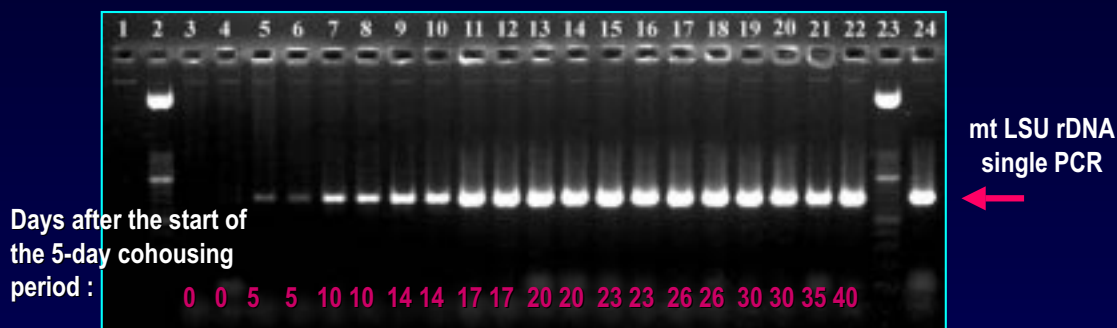
Results - 1



Microscopic detection of cysts in lung smears of a healthy mouse 28 days after the end of 5-day cohousing period

Chabé et al, 2004. Eur J Clin Med Infect Dis (in press)

Results - 2



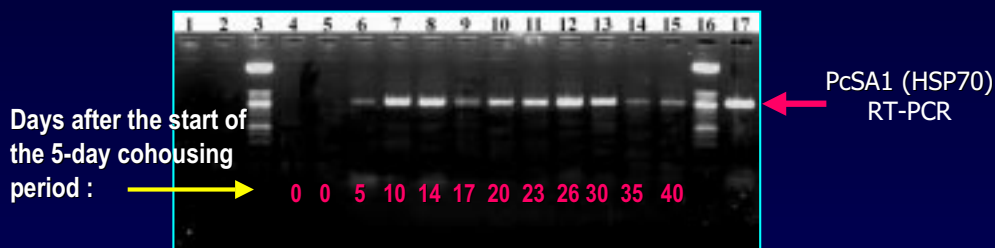
Molecular detection : lung samples from all Balb/c mice co-housed with infected donor SCID mice for 1 or 5 days yield positive first or second round PCR results

Molecular identification : *Pneumocystis carinii* f.sp. *muris*

Pneumocystis serum antibodies : from the 17th day after the start of the 5-day cohousing

Chabé et al, 2004. Eur J Clin Med Infect Dis (in press)

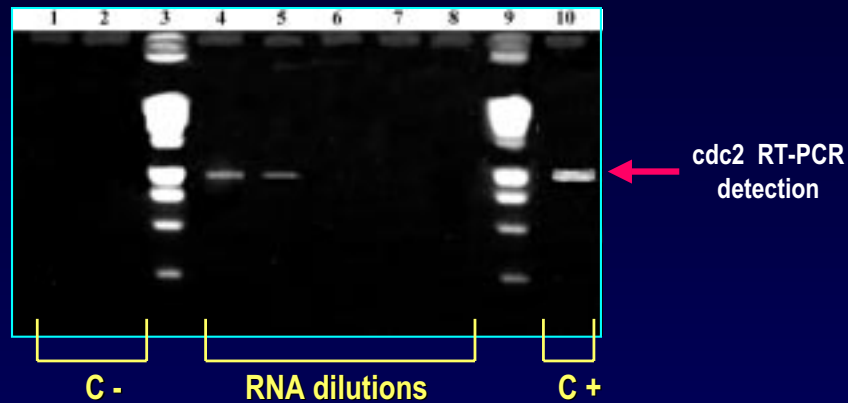
Results - 3



Expression of *Pneumocystis* HSP70 gene in healthy carriers : 5 - 40 days after the start of the 5-day cohousing period

Chabé et al, 2004. Eur J Clin Med Infect Dis (in press)

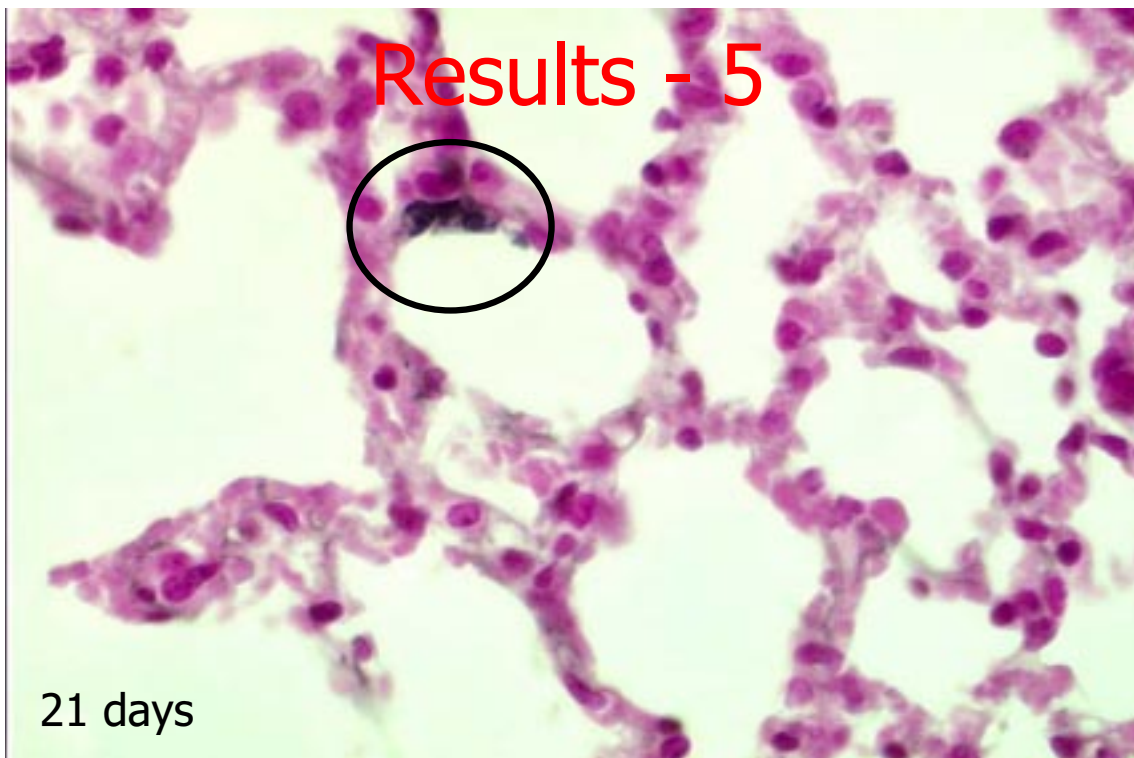
Results - 4



Expression of *Pneumocystis cdc2* gene in healthy carriers : 5, 11 or 26 days after the start of the 5-day cohousing period

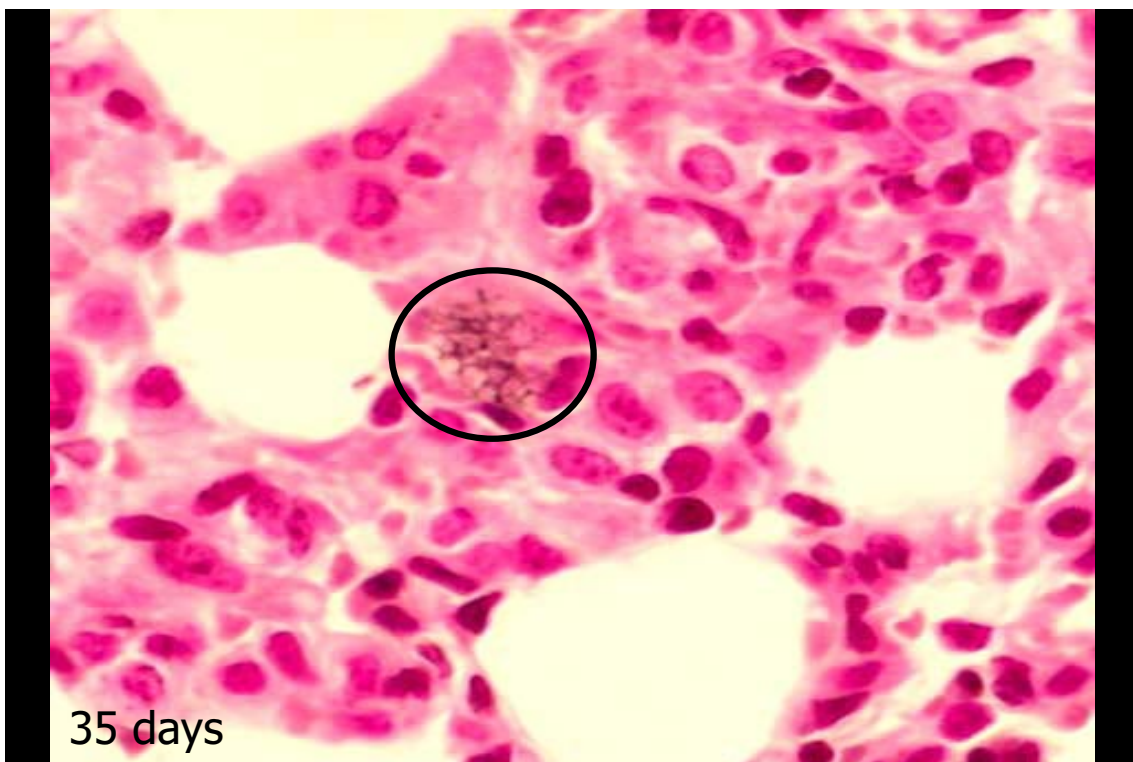
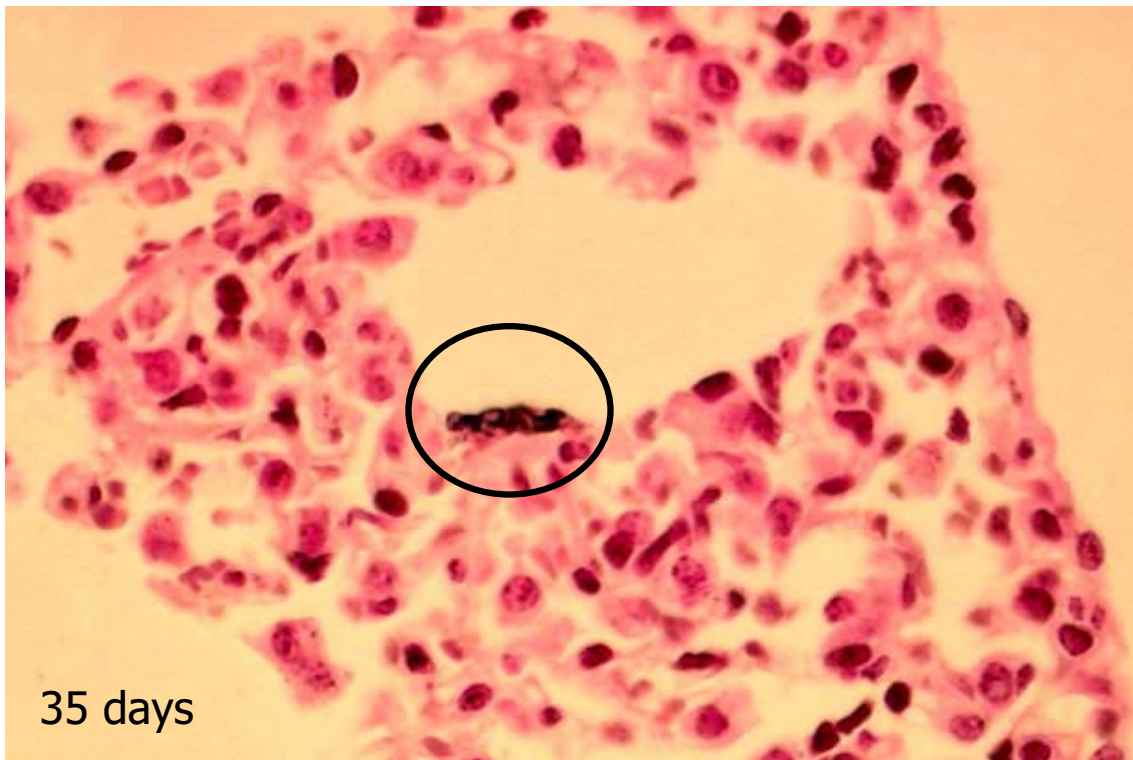
Chabé et al, 2004. Eur J Clin Med Infect Dis (in press)

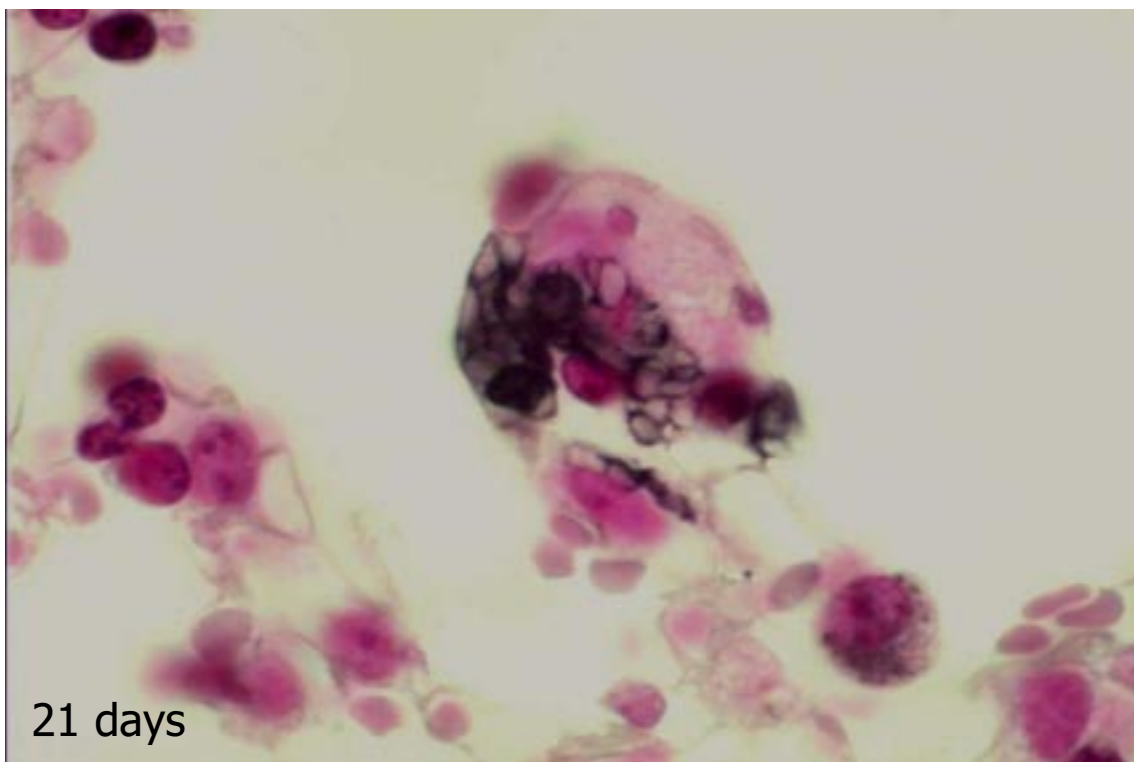
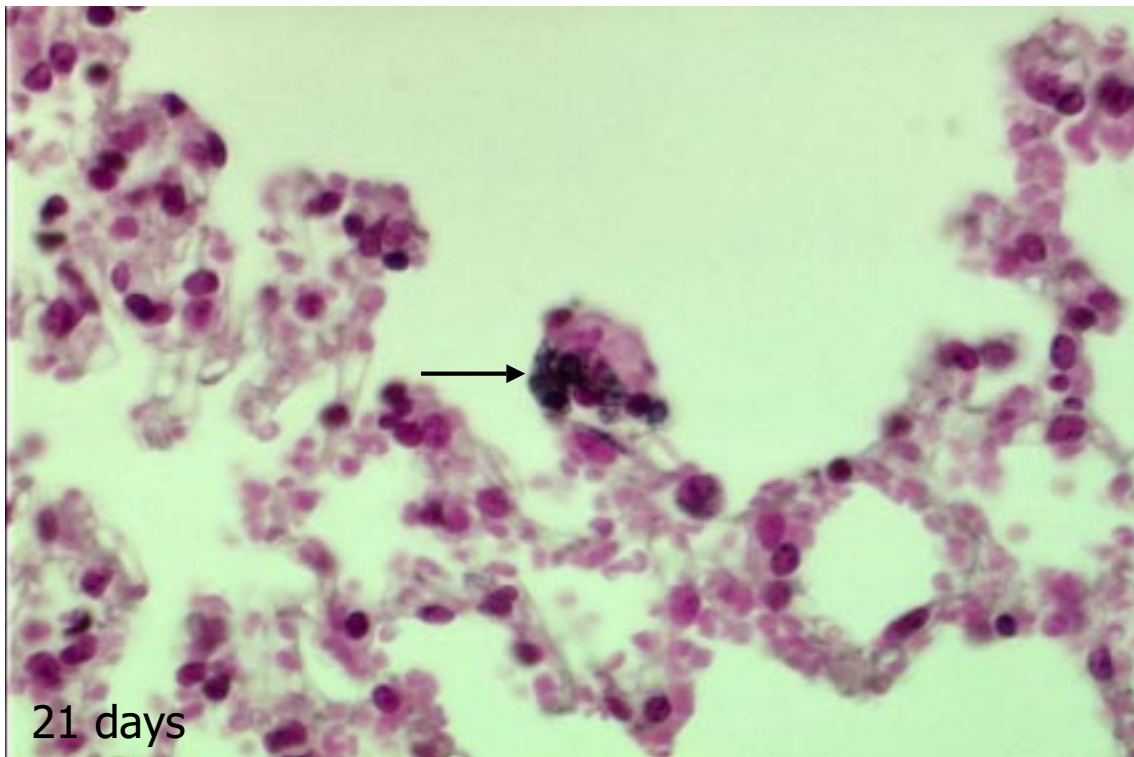
Results - 5



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WORKSHOP II





Comments

- Healthy hosts are able for eliminating radically the parasites from their lungs; but as long as they remain infected, they could transmit the infection:
 - to immunocompetent hosts, who will develop a primary infection, like infants, or a transient reinfection in other cases
 - to immunosuppressed hosts, able to develop clinical PcP, and by this way, to amplify the *Pneumocystis* population

Chabé et al, 2004. Eur J Clin Med Infect Dis (in press)

Conclusion: a hypothesis of *Pneumocystis* reservoir

- Immunocompetent hosts play a role of *Pneumocystis* **dynamic reservoir** :
 - more or less susceptible to the infection, they permit a time-limited *Pneumocystis* replication in their lungs, the production of infective forms and the airborne transmission of the infection to other members of the population

Acknowledgment

- European 'Eurocarinii' network (FP-5, QLK2 CT2000 O1369)
- French network of 'Pneumocystis Research', supported by the Ministry of Research
- National Agency to AIDS Research (ANRS) has supported in part this work
- Careworkers of the Lille-2 University Hospital, who have participated to this study

Pneumocystis Primary Infection in children: Seroepidemiological data

n =	Age	Test	Antigen	Prevalence (%)	Region	Reference
44	24 months	IFA	<i>P. jirovecii?</i>	65.0 (IgG) 95.0 (IgM)	Holland	Meuwissen et al 1977
33	24 months	IFA	<i>P. carinii</i>	50.0 (IgG)	USA	Pifer et al 1978
15	0-24 months	IFA	<i>P. jirovecii?</i>	53.0 (IgG)	USA	Gerrard et al 1987
73	0-24 months	WB	<i>P. jirovecii?</i>	82.0 (IgG)	USA	Peglow et al 1990
150	8 years	ELISA	<i>P. carinii</i>	70.0 (IgG)	Gambia/ UK	Wakefield et al 1990
472	0-24 months	ELISA	<i>P. carinii</i>	72.5 (IgG)	Korea	Hong et al 1991
40	0-24 months	ELISA	<i>P. jirovecii</i>	30.0 (IgG) 00.0 (IgM)	Denmark	Lundgren et al 1993
15	0-24 months	WB	<i>P. carinii</i>	40.0 (IgG)	Korea	Moon et al 1995
79	0-20 months	ELISA	<i>P. muris</i>	84.5 (Ig)	Chile	Vargas et al 2001
133	4 – 13 years	WB	<i>P. carinii</i>	70.3 (Ig)	Spain	Calderon et al 2003 (unpublished)

Totet A 2003 'Thesis' Dissertation (modified)

P. jirovecii in Infants with Bronchiolitis

Pneumocystis mtLSU rRNA nested PCR on nasopharyngeal aspirate samples from infants of 4.7 months (2 – 12 months)

Data	n =	<i>Pneumocystis jirovecii</i>	P
Age: 2-12	174	46 (26.4%)	0.04
12-24	11	0 (0.0%)	
Urban habitat	93	15 (15.1%)	< 0.01
Rural habitat	90	31 (32.6%)	
Body weight at birth: 10 – 25 percentil	26	11 (42.3%)	0.03
<10 >25 percentil	138	30 (21.7%)	

Nevez et al, *J Euk Microbiol* 2001, 48(Suppl), 122-3

The Methodological Approach

- Using a noninvasive sampling method:

Oropharyngeal wash sampling (OW)

- rinse and gargle 30 sec to >1min with 10ml of sterile saline (0.9%) >4h after eating or washing of the mouth or teeth;
- transport rapidly on ice to the laboratory;
- divide in 2 aliquots and store the pellet at –20°C.

Wakefield et al. 1993. *Q J Med* 86: 401-6

Pneumocystis DNA amplification and identification

- nested-PCR at mt LSU rRNA locus

Wakefield A.E. 1990. *Lancet* 336 : 451-3; Wakefield A.E. 1996. *JCM* 34: 1754-59; Tsolaki et al. 1999. *JMM* 48: 897-05

- direct sequencing and / or hybridization with an oligonucleotidic probe specific to *P. jirovecii*

Durand-Joly I et al. 2002. *J CM* 40:1862-5

Postvaccine Meningopolyradiculoneuritis Caused by Polio Virus Type 2 and 3 – A Case Review

Vesna Turkulov

E2

WORKSHOP II

The objective of this study is to show a case of a three year old child with the diagnoses of meningopolyradiculoneuritis that occurred after the vaccination against the child paralyzes.

The child had been admitted to the Clinic for Infectious Diseases on the 4th day of illness, which had been manifested by increased temperature and dozing, while weakness and disability to move the left arm occurred on the day of the admission. We had anamnestically obtained the information about receiving the first dose of the Polio vaccine, three weeks before the onset of the disease. The child had not been vaccinated before because of eczema. During that same period, a tick had bitten the child. Two days after the vaccination, a short termed diarrhea had occurred.

On admission, the child had been afebrile, somnolent, with exhibited meningeal signs, limp paralysis of the left arm, with no tendon reflexes in the right arm, and with diminished sensibility. Clear cerebrospinal fluid (CSF) had been obtained by lumbar puncture. The cytochemical findings in the cerebrospinal fluid corresponded to serous meningitis. From the patient's stool, the vaccine species of Poliovirus type 2 and 3 had been isolated. The immunofluorescent test of blood and CSF to the *Lyme borreliosis* excluded the possibility of the Lyme disease.

The corticosteroid and immunoglobulin therapy did not yield significant improvement of the neurological findings; hence the child was sent to the physical treatment.

Postvaccine encephalomyelitis is seldom encountered, but one should be cautious when the primovaccine is administered later than usual calendar of the vaccination suggests, especially in children with exhibited hypersensitive allergic reactions.

PRESTO – Prevention Study of Obesity

A project to prevent obesity during infancy and adolescence

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Background

Data from Vienna showed that 12 - 22% of children between the age of 10 and 15 years are overweight, 5-10% are obese and up to 2% morbid obese. A school-oriented research project (PRESTO) has been launched due to the obvious necessity of preventing overweight and obesity in children and adolescents.

Objectives

- Primarily the prevention of obesity during infancy and adolescence
- Early registration and therapy of overweight and obese children and adolescents – identification of patients at risk
- Education about the development of obesity and conveyance of strategies to prevent overweight
- Better prevention and health care for children and adolescents within the bounds of existing health care structures
- Promotion of health-oriented eating habits

Methods

PRESTO is a pilot project in Austria. Initially it was carried out in 5 first grade grammar and 5 first grade comprehensive school classes in Vienna, in one first grade grammar school class in Waidhofen/Ybbs and in one first grade comprehensive school class in Molln. The same number of classes served as a control group.

The school-oriented intervention was performed by a multi-professional team (physician, psychologist, nutritionist, expert for physical activity). At the beginning a school medical examination was conducted in cooperation with the school medical officer. A precise family anamnesis should give information about risk factors and influences on developing obesity during infancy and point out possibilities for a suitable intervention. By means of a quiz the children's knowledge of nutrition as well as eating habits were evaluated before and after the intervention.

The intervention covered 11 nutrition related sessions, one hour per week in each class. Additionally, teachers should include themes of nutrition and health in their subjects suitably to the curriculum. Physical education teachers should arrange their lessons in such a way that also overweight children can participate without any problems and that they can keep up with "normal weight" children. Appropriate exercises and sessions were shown on a common day of physical activity.

The project is going on for 2 years. The intervention was followed up by careful checking on a short term basis and will also be followed up on long term basis.

Conclusion

PRESTO is designed to answer the following questions: Can school intervention influence the fields of nutrition, physical activity and health? How largely is the increase of knowledge immediately after the intervention? Is this of short-term and long-term duration? Does knowledge mean conversion?

After successful completion and evaluation PRESTO should be expanded onto other schools throughout Austria.

Skin and infectious diseases in immigrant children

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E2

WORKSHOP II

It is vital to build a space where our children and the children of immigrants can meet as equals. We continue to speak of them as 'immigrants', even though two thirds of them did not come to Italy but were born here. While the immigrant population has doubled during the last 10 years, for minors this has happened in just 4 years. Their numbers increased from 126,000 at the end of 1996 to 278,000 at the end of 2000. Including new births (more than 25,000) and those entering through family reunion, their number already exceeds 300,000, a fifth of the immigrant population.

The term "bambino straniero" (foreign child) is also incorrect, because we are often talking of children born in Italy, who talk like us, have the same tastes and can often be distinguished only by facial characteristics. Their number reached 100,000 only four years ago, and grew to 147,000 during the school year 2001-2002 and 182,000 in the following year. Six out of ten are enrolled at primary or nursery schools. They are now less than 2% of the resident population; in 2017, according to a government estimate, this could rise to 529,000, or 6.5% of the school population.

An investigation of schools throughout Italy by the Ministry of Education in 2001, found that in only 7% of schools there are no foreign pupils (the percentage is about three times higher in the South), in 64% foreign children make up more than 3% of the school population, and in 28% more than 5%. This population is very varied in relation to country of origin, and is highest in primary and comprehensive schools.

Migration policy focuses a great deal of attention on immigration flows, which is understandable since the newly arrived are, in a manner of speaking, the valve which regulates the growth of the foreign population. It should not, however, ignore settled immigration and particularly long-established immigration, since it now represents the majority of immigration and expresses the new social reality in the host country. To be concerned solely with new arrivals is to confine ourselves to emergency measures, ignoring the more profound needs for coexistence. Cultural mediation is very important in this context as a way of integrating the first generation, and even more important, the second generation.

In 2001 the number of foreign children in Italy was 280,000 and the 49% of them was born in Italy which is now the oldest Nation in the world with the 24,5% of over 60 people.

For the first time in the history of human beings, the number of over 60 people will exceed, in 2050, the number of under 14 with very important economic and social consequences related to this demographic disequilibrium.

We visited 1,137 foreign minors in the last three years (401 from East-Europe, 185 from Latin-America, 156 from Asia and 140 from Africa), 255 of them had the double nationality and 50% of them were under 14. We observed a high prevalence of odontoiatric diseases (50.3%), skin diseases (24.4% scabies, 20.1% pediculosis, 9.6% tinea capitis, 8.9% tinea corporis), traumatic diseases (10.2%) and gastroenteric diseases (6.2% gastritis and duodenitis, 6.1% liver diseases) and also skin tuberculosis and HIV. Many little girls are affected by different forms of Female Genital Mutilation. We examined 654 abandoned children in the last five years. They are often victims of various kinds

of exploitation (theft, illicit trading, mendacity, etc.). They come to Italy without their families and for this reason they are easy victims.

E2

In our experience the health condition of foreign minors in Italy is still worse than that of the Italian minors and there are not enough epidemiological and clinical studies in this field.

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Visceral Leishmaniasis:

A Neglected Public Health Threat

Presented by
Fahad A. AL-Ateeg, MHA, M.Ed., Dr. P.H.
Genoa January, 2004

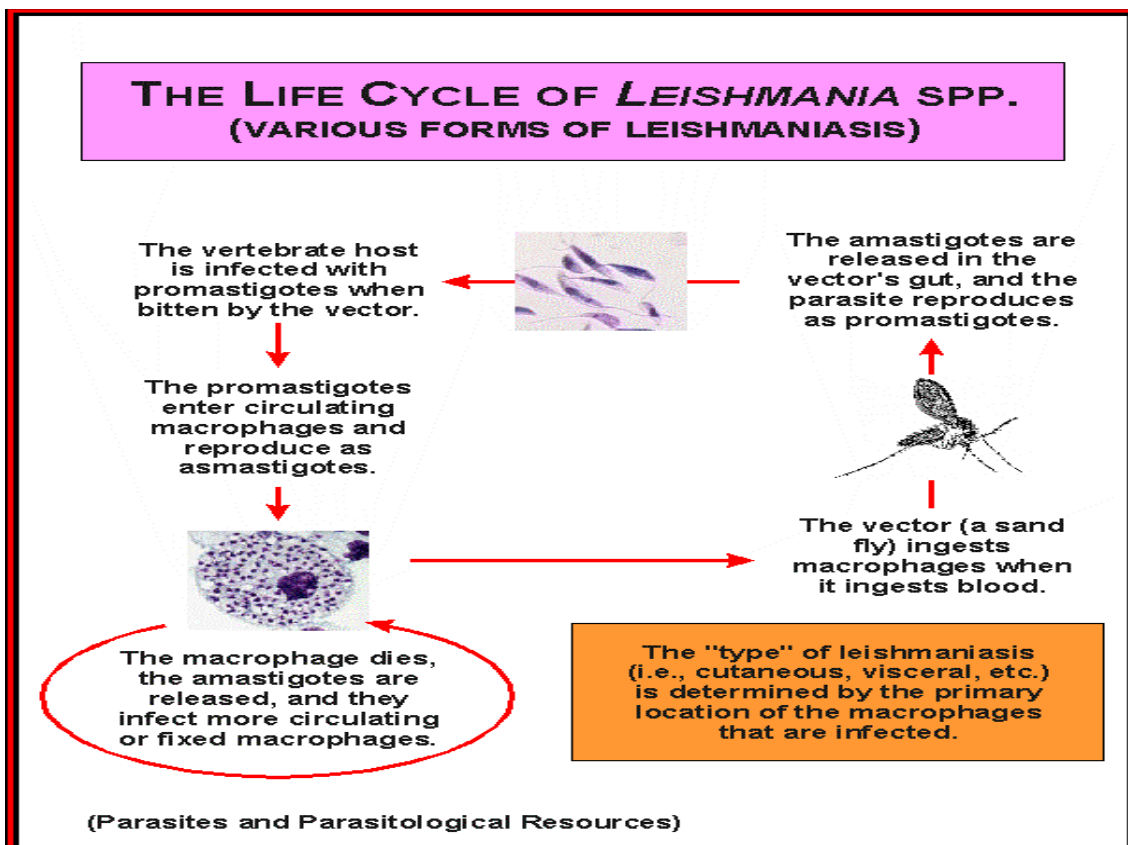
Introduction

- Ä The leishmaniasis are group of parasitic diseases caused by protozoa of the genus leishmania.
- Ä It was first described in 1903 by Leishman and Donovan.
- Ä It is transmitted through the bites of infected female ***phlebotomine*** sandfly.

Vector



3



The Burden of the Disease

- Ä The disease is found in five continents-Africa, Asia, Europe, North America & South America.
- Ä It is endemic in more than 88 countries, 72 of which are developing countries.
- Ä The true incidence is not known.
- Ä Approximately 12 million people throughout the world are currently infected.
- Ä 350 million individuals are at risk worldwide.
- Ä Incidence is highest in rural areas and areas where conditions are favorable for sandflies.

5

Primary Causes for Increased Exposure to the sandfly

- Ä Man-made environmental changes (such as, extracting timber, mining, building dams, new irrigation scheme).
- Ä Expanding road construction in primary forests such as the Amazon.
- Ä Widespread migration from rural to urban areas.
- Ä Continuing fast urbanization worldwide.

6

Major Clinical Syndromes

- Ä Cutaneous/Dermal Leishmaniasis (CL)
- Ä Mucocutaneous Leishmaniasis (MCL)
- Ä Visceral Leishmaniasis (VL)
- Ä These forms are produced by the interaction of the parasite and the immunological conditions of the infected person.

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Visceral Leishmaniasis

- Ä The most severe form of leishmaniasis.
- Ä It is a disease of public health importance in most regions bordering the Mediterranean Sea, including Central and southern Italy, Sardinia, and Sicily.
- Ä It attacks the spleen, liver and lymph.
- Ä The clinical form most frequently associated with HIV/AIDS.
- Ä It is usually fatal if left untreated.

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Etiology

Ä *L donovani*

- Ä The primary cause of VL in the Indian subcontinent and East Africa.
- Ä Predominates in Indian children between 5 and 15 years of age.

Ä *L infantum*

- Ä The Mediterranean region.
- Ä It infects infants and young children 1 to 4 years.
- Ä Dogs, foxes, or feral animals are the reservoirs.

Ä *L chagasi* (in the new world).

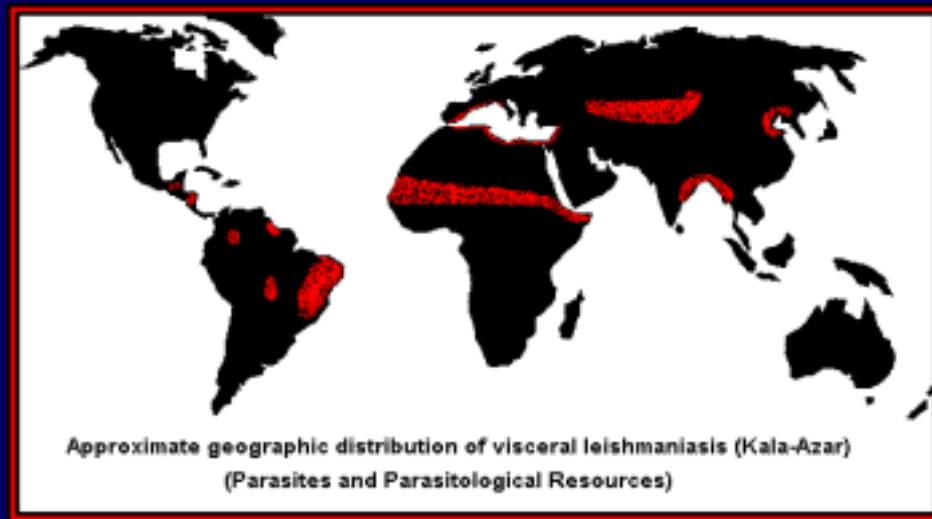
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Epidemiology

- Ä Endemic in 62 countries.
- Ä A total of 200 million people at risk.
- Ä Estimated incidence is 500,000 cases per year.
- Ä 90% of these cases are found in Bangladesh, Brazil, India, Nepal and Sudan.
- Ä There are 30-100 subclinical infections for every overt case of VL.
- Ä 41,000 recorded death in the year 2000.
- Ä Males are infected more often than females.

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Geographic Distribution



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Risk Factors

- Ä Children are at greater risk than adults in endemic areas.
- Ä Malnutrition has been shown to contribute to the development of the disease.
- Ä Persons with AIDS have an increased risk of developing VL.
- Ä Incomplete therapy of initial disease is a risk factor for recurrence of leishmaniasis.

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Common Clinical Presentation

Ä Subclinical.

Ä Acute Presentation:

- Ä Fever.
- Ä Cough.
- Ä Abdominal Pain.
- Ä Diarrhea.
- Ä Epistaxis.
- Ä Pancytopenia.

Ä Chronic

Presentation:

- Ä Prolonged Fever.
- Ä Poor Appetite.
- Ä Weight Loss.
- Ä FTT.
- Ä Hepatosplenomegaly.
- Ä Lymphadenopathy.
- Ä Darkening of skin.

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VL & HIV Co-infection

- Ä VL is an important opportunistic infection in patients infected with HIV.
- Ä Co-infections have been reported in 33 countries worldwide.
- Ä The clinical features are similar to those of classic disease.
- Ä They are not always present.
- Ä May be hidden by other associated opportunistic infections with similar symptoms.

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VL & HIV Co-infection Cont...

- Ä Unusual presentation may occur.
- Ä Reactivation of asymptomatic or previously healed infections is common.
- Ä Persons with AIDS may serve as a reservoir for human-sandfly-human transmission.
- Ä Co-infected patients may be difficult to diagnose, respond poorly to treatment, and relapse repeatedly.

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Diagnosis

- Ä CBC:
 - Ä Anemia (Hb =5-8 mg/dl).
 - Ä Thrombocytopenia.
 - Ä Leukopenia (2000-3000 cells/ml).
- Ä Liver Function Test:
 - Ä Increased hepatic transaminase level.
- Ä Hyperglobulinemia (> 5gm/dl).
- Ä Serological:
 - Ä Indirect fluorescent antibody test (IFAT).
 - Ä Direct agglutination test (DAT).
 - Ä Enzyme linked immunosorbent assay (ELISA).
 - Ä Formal gel test.

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Diagnosis

- Ä Parasitological:
 - Ä Blood Buffy Coat & Culture.
 - Ä Bone Marrow.
 - Ä Lymph Node.
 - Ä Spleen.
- Ä Molecular Probes using:
 - Ä Kinetoplast DNA (kDNA).
 - Ä Ribosomal RNA (rRNA).
 - Ä Mini exon derived RNA (medRNA).
 - Ä Genomic repeats.

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Prevention

- Ä Use of fine mesh screens impregnated with insecticides such as permethrin to avoid sandflies bite.
- Ä Use of repellents such as diethyltoluamide (DEET) applied to the exposed area of the body and clothing.
- Ä Elimination of sandfly vectors through large scale spraying with chemicals such as DDT.
- Ä Control and elimination of species that act as protozoal reservoirs such as dogs.

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Treatment

Drug	Trade Name	Regimen	Considerations
Pentavalent antimonials	Pentostam Stibanate Stibogluconate Glucantime	20 mg/kg daily for 20-40 days intravenously	First-line agents. Resistance, toxicity in HIV co-infection and long courses.
Potential Side effects			
Nausea, vomiting, diarrhea, ECG changes and convulsions. However, some of the side effects are identical with features of the disease.			

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Alternative Treatment

Drug	Potential Side effects
Pentamidine Lomidine	The toxic side effects include a sensation of burning, headache, tightness of the chest, dizziness, nausea,, vomiting, hypotension, hypoglycaemia, hyperglycaemia and acute pancreatitis.
Amphotericin B	Anaphylaxis, thrombocytopenia, flushing, generalized pain, chills, fever, phlebitis, anaemia, convulsions, amorexia, decreased renal tubular & glomerular function, and hypokalaemia.

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Alternative Treatment Cont...

Drug	Potential Side effects
Lipid-associated amphotericin B	Chills, fever, increased respiratory rate and acute febrile reaction in 80% of the patients during infusion.
Interferon gamma (INF-gamma)	Produce only minor side effects which are usual for cytokine therapy. Fever and flu-like symptoms are common side effects.
Miltefosine	Teratogen.

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Complications

- Ä Secondary Bacterial Infection.
- Ä Hemorrhage.
- Ä Severe Anemia.
- Ä Hypersplenism & Splenic Rupture.
- Ä Progressive Emaciation.
- Ä Post Kala-azar Dermal Leishmaniasis.

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Prognosis

- Ä In children and immuno-compromised adults, the disease can be devastating.
- Ä Fatal in 75-85% of infantile cases & 90% of adult cases.
- Ä Properly treated at an early age, 85-90% of cases can be cured.
- Ä Patients with pancytopenia or bleeding diatheses or who fail to develop a suitable cell-mediated immunologic response to infection is usually poor.
- Ä In endemic area, mortality rate can be as high as 10%.
- Ä Refractory cases and clinical relapses are not uncommon.

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How VL Is Being Neglected?

- Ä After more than a century of its discovery in 1900, we still do not have an effective vaccine.
- Ä No real promising prospects of the development of one in the near future.
- Ä The substantial knowledge of parasite biology has not been translated into an effective and affordable drug.
- Ä An examination of the current available drugs indicates that the therapeutic arsenal against VL is limited in their potential to control the disease.

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How VL Is Being Neglected?

- Ä The available agents with established efficacy are all injectable and require parenteral administration for one month or more.
- Ä The parasite has developed resistance to antimony, the mainstay treatment for over 60 years, in several endemic areas.
- Ä The use of second line drug pentamidine, a toxic drug with declining efficacy, has been largely abandoned.
- Ä The present high costs of the three lipid associated new formulations makes them of almost no practical value in less developed countries.

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How VL Is Being Neglected?

- Ä Miltefosine, the first oral compound, is considered to be one of the rare new treatments that have been available in recent decades and it is still in the experimentation phase.
- Ä No regular global surveillance exists.
- Ä Leishmaniasis is notifiable in only 33 out of the 88 countries where it occurs.
- Ä The current surveillance network is limited to the leishmania/HIV co-infection and coverage is still incomplete .
- Ä VL is not recognized as an official opportunistic infection which means that it is not reported in HIV/AIDS notification system.

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Conclusion Remarks

- Ä VL is a disease of public health importance.
- Ä It is one of several diseases that takes a huge toll on people in developing countries.
- Ä However, it receives little attention from the western world.
- Ä Prevention and control requires systematic surveillance.
- Ä Surveillance should include the human host, the reservoir host and the vector.

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Conclusion Remarks Cont...

- Ä Surveillance of the human host must be based on both active & passive detection of cases.
- Ä Active detection of cases requires diagnostic facilities in endemic areas.
- Ä Passive detection relies on the awareness of the people and physicians of the early symptoms of the disease.
- Ä Both passive & active detections of human cases are important in the anthroponotic cause of VL (*L. donovani*).

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Conclusion Remarks Cont...

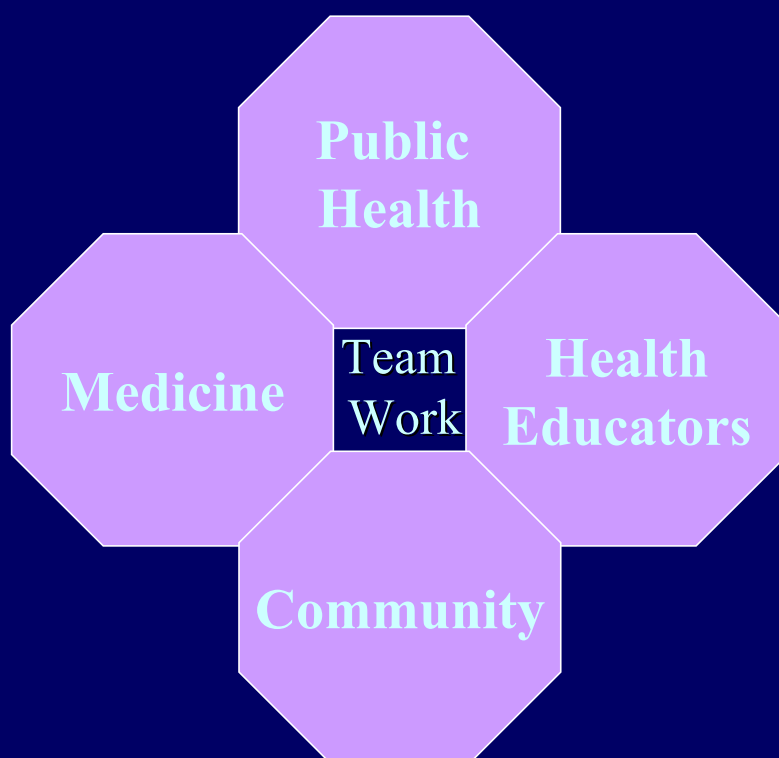
- Ä In the zoonotic cause of VL (*L. infantum*), early detection helps to reduce morbidity and mortality but does not help to reduce transmission except in the case of VL/HIV co-infection.
- Ä Therefore, surveillance of animal reservoir host(s) in the zoonotic cause of VL is a prerequisite.
- Ä The evolution of VL/HIV co-infection must be monitored closely by extending the geographic coverage of the surveillance network.
- Ä Finally, drug development should not be left to the private industry alone.

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Conclusion Remarks Cont...

- Ä Out of the 1,393 new drugs developed between 1975 and 1999, only 16 were targeted for tropical diseases and tuberculosis.
- Ä Therefore, an international pharmaceutical policy for neglected diseases is required .
- Ä More emphasis and investigation needs to go to obliging the private sector to invest in diseases of the poor.
- Ä Non-for-profit drug development initiatives need to be explored.

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Team Work



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