



**Promising antiprotozoal
activities of bee propolis**

Propolis



Propolis is a natural mixture produced by honeybees from substances collected from parts of plants, buds, and . exudates

Propolis is used by bees in the construction and repair of their hives

for sealing openings and cracks

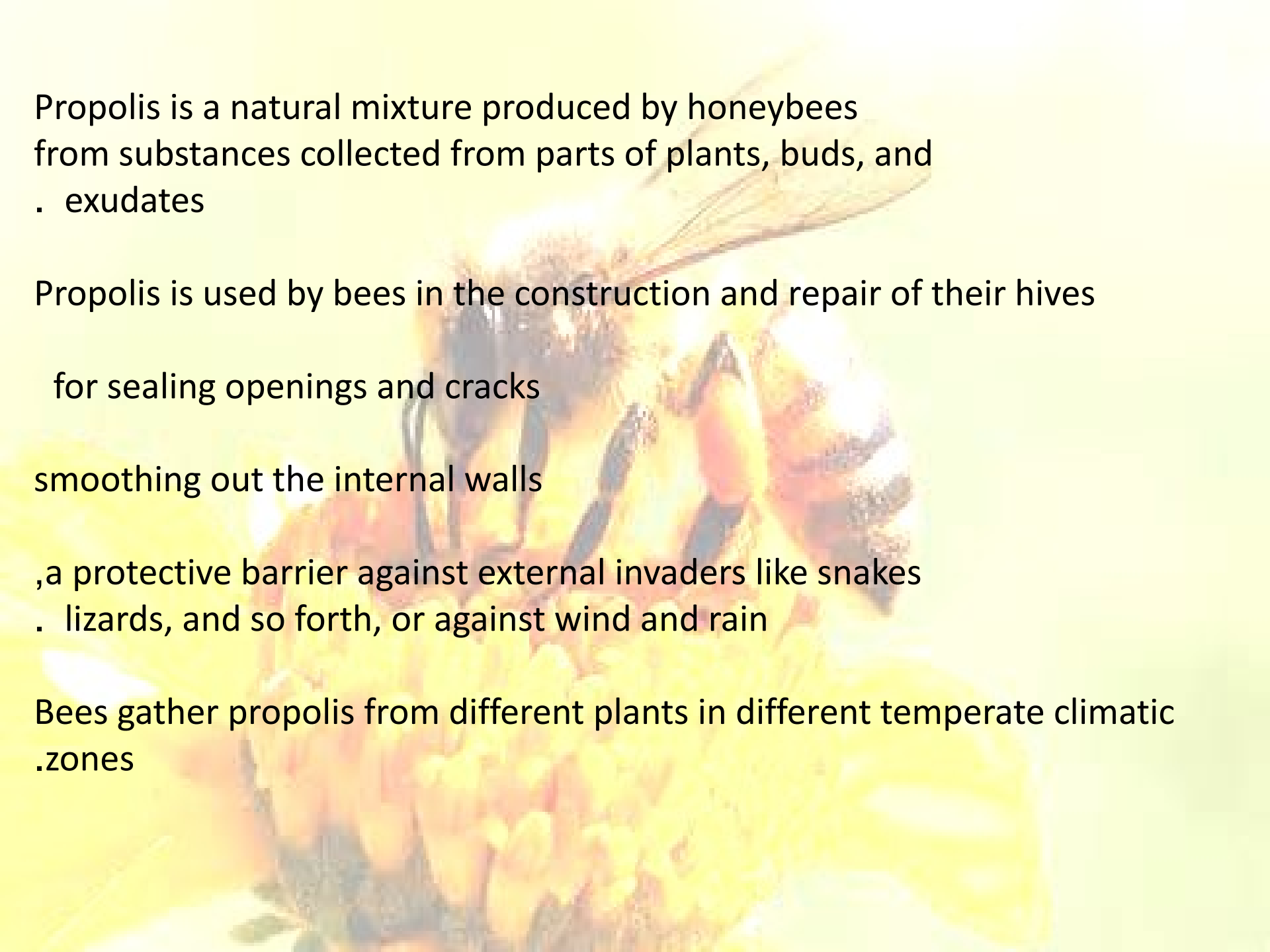
smoothing out the internal walls

,a protective barrier against external invaders like snakes

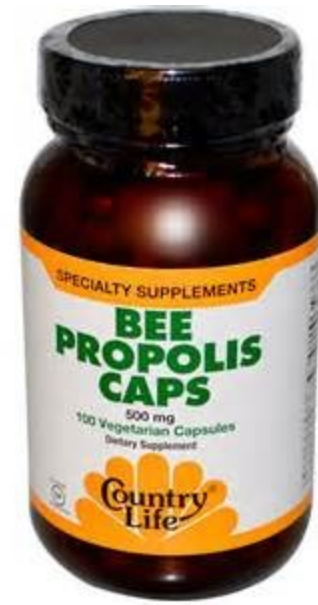
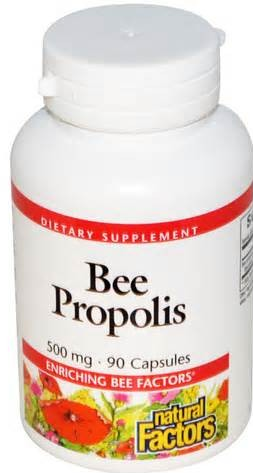
. lizards, and so forth, or against wind and rain

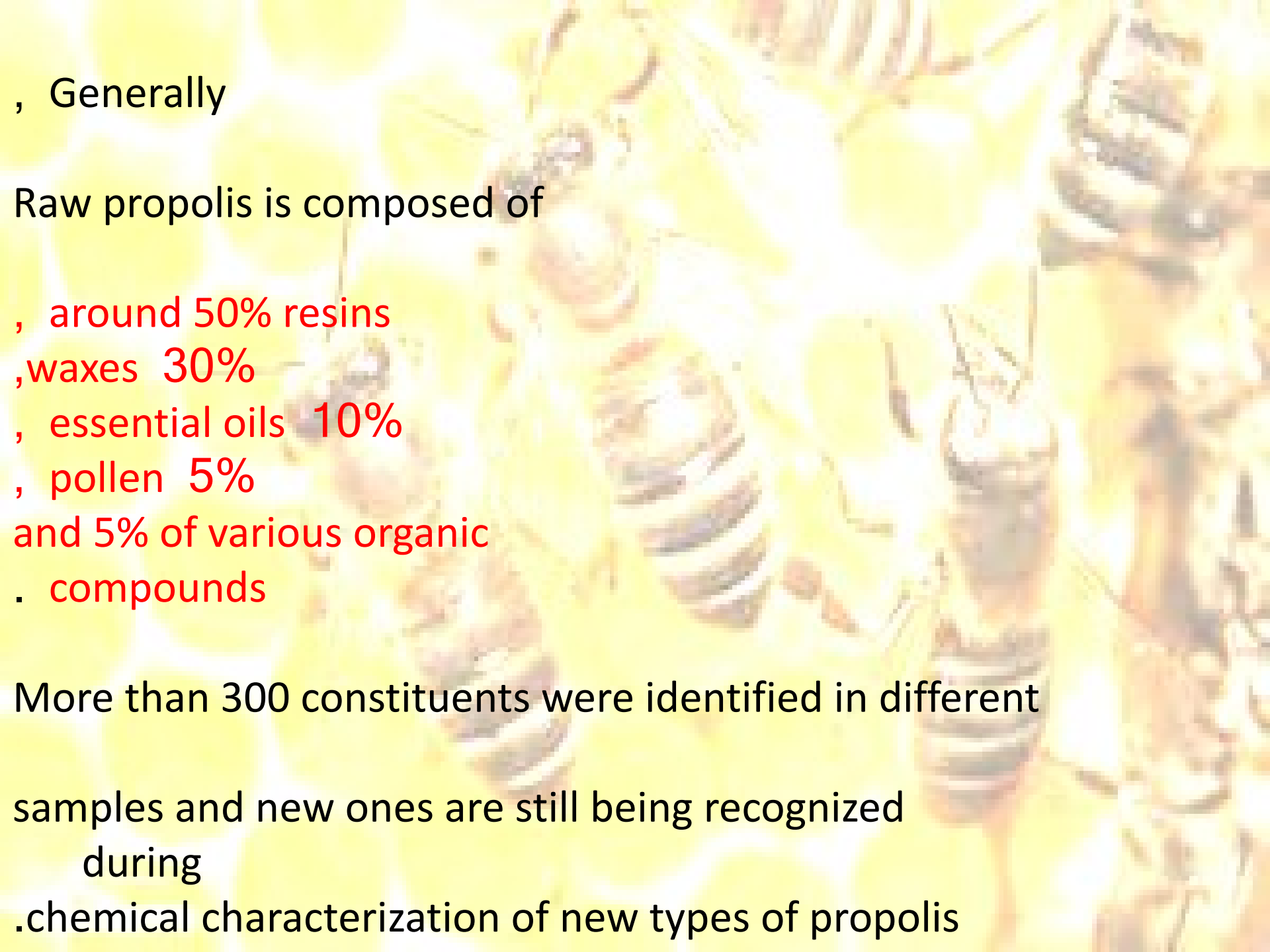
Bees gather propolis from different plants in different temperate climatic

.zones



Pharmaceutical products of propolis





, Generally

Raw propolis is composed of

- , around 50% resins
- , waxes 30%
- , essential oils 10%
- , pollen 5%
- and 5% of various organic
· compounds

More than 300 constituents were identified in different
samples and new ones are still being recognized
during
· chemical characterization of new types of propolis

Malaria



Malaria

The disease is widespread in the tropical and subtropical regions that exist in a broad band around the equator which includes much of Sub-Saharan Africa, Asia, and Latin America.

In 2015, there were 214 million cases of malaria worldwide resulting in an estimated 438,000 deaths, 90% of which occurred in Africa.

Rates of disease have decreased from 2000 to 2015 by 37%,but increased from 2014 during which there were 198 million cases.

In Africa, it is estimated to result in losses of US\$12 billion a year due to increased healthcare costs, lost ability to work, and negative effects on tourism.

Symptoms of **Malaria**

Central

- Headache

Systemic

- Fever

Muscular

- Fatigue
- Pain

Back

- Pain

Skin

- Chills
- Sweating

Respiratory

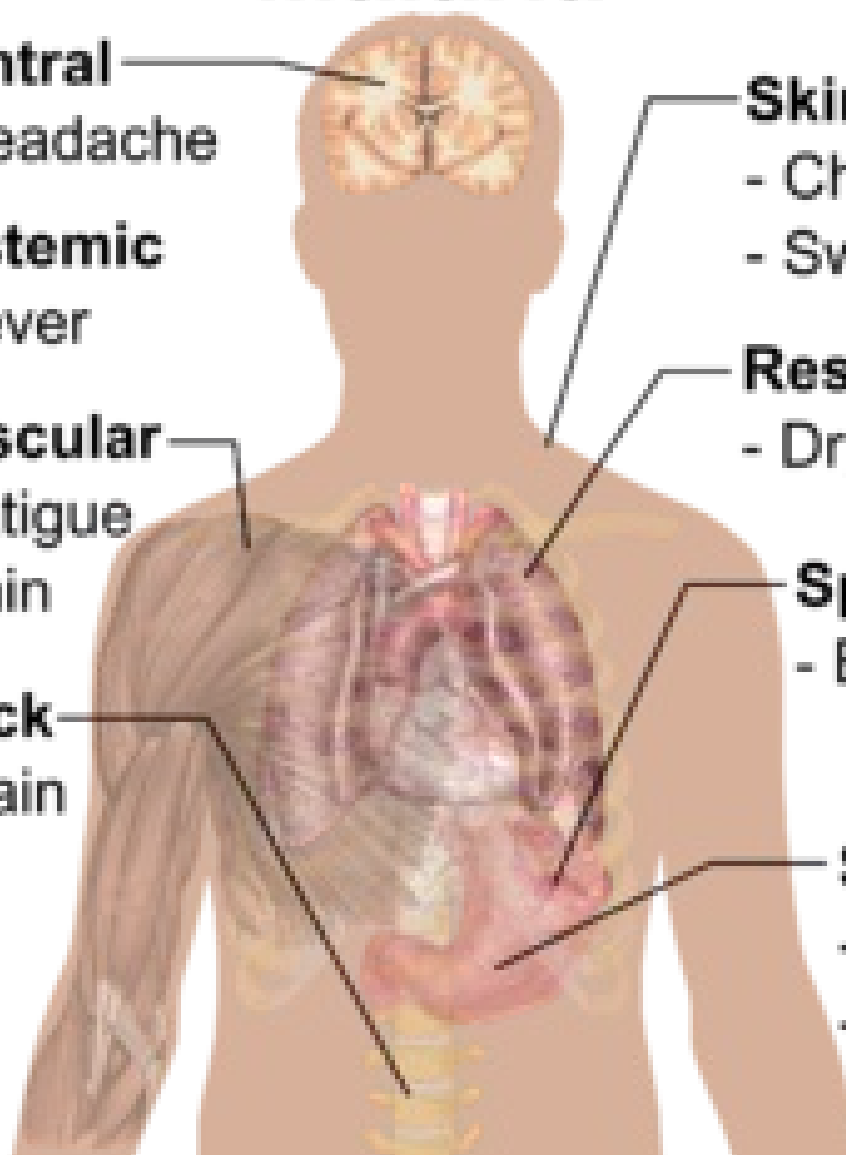
- Dry cough

Spleen

- Enlarge-
ment

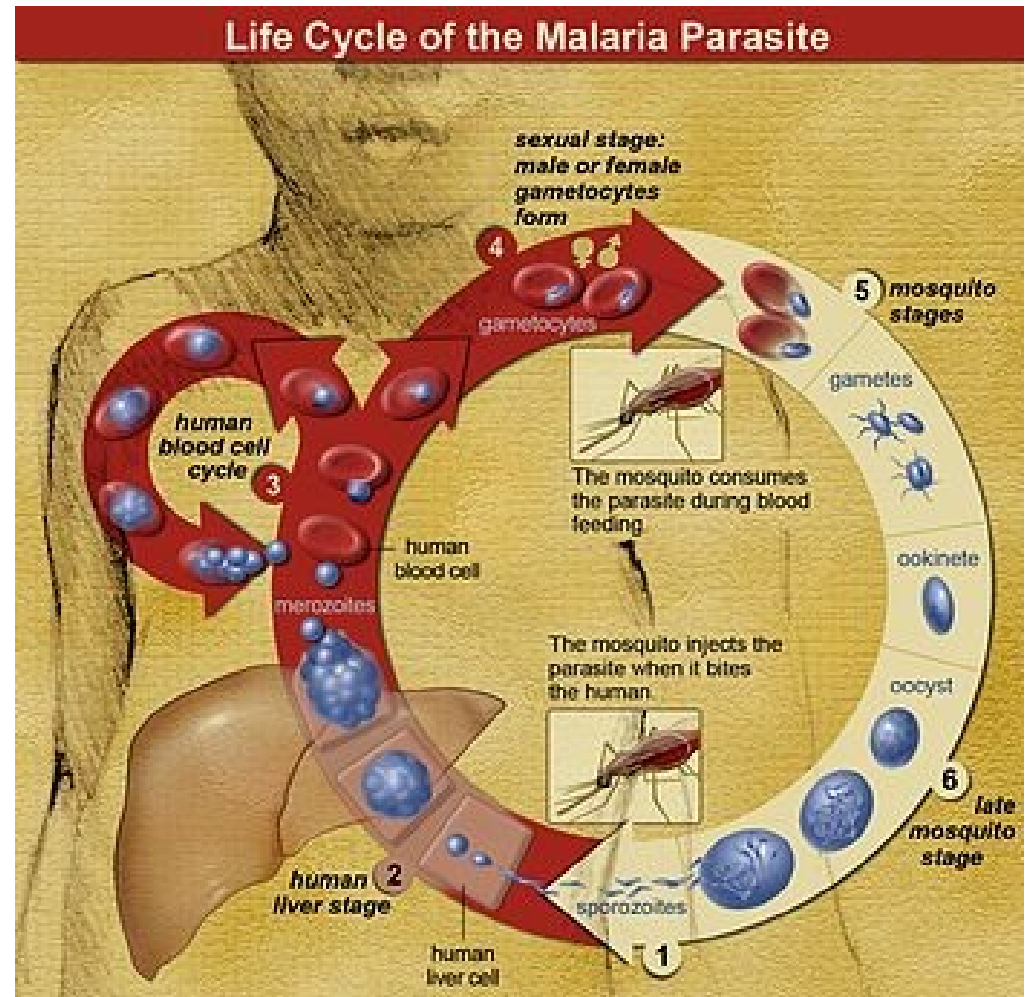
Stomach

- Nausea
- Vomiting



Life Cycle

The life cycle of malaria parasites. A mosquito causes an infection by a bite. First, sporozoites enter the bloodstream, and migrate to the liver. They infect liver cells, where they multiply into merozoites, rupture the liver cells, and return to the bloodstream. The merozoites infect red blood cells, where they develop into ring forms, trophozoites and schizonts that in turn produce further merozoites. Sexual forms are also produced, which, if taken up by a mosquito, will infect the insect and continue the life cycle.



Antimalarial effect of propolis

- In vivo evaluation of antimalarial effect in albino mice were used
PE significantly suppressed the parasitaemia and showed significant efficacy in reducing anaemic conditions in *P. chabaudi*-infected mice in a dose-dependent manner (25, 50 and 100 mg PE/kg).

Antimalarial effect of propolis

**Histological investigation of the spleen •
tissue of treated and untreated mice further
supports the antimalarial potential of PE.
In addition, our study proved that Saudi PE
reduced oxidative damage by decreasing the
malondialdehyde (MDA) and increasing the
catalase (CAT) activity and the glutathione
· (GSH) levels**

Antimalarial effect of propolis

Also, Saudi PE increased the level of some •
pro-inflammatory cytokines such as IFN- γ ,
TNF- α ,.

In conclusion, PE showed antimalarial and
antioxidant activities and provided
protection against spleen tissue damage in *P.*
.chabaudi-infected mice

Leishmania

***Leishmaniasis* currently affects 12
million people in 98 countries
About 2 million new cases occur
, each year
species are known to cause 21
disease in humans**

Leishmania

Phylum Sarcomastigophora

Order Kinetoplastida

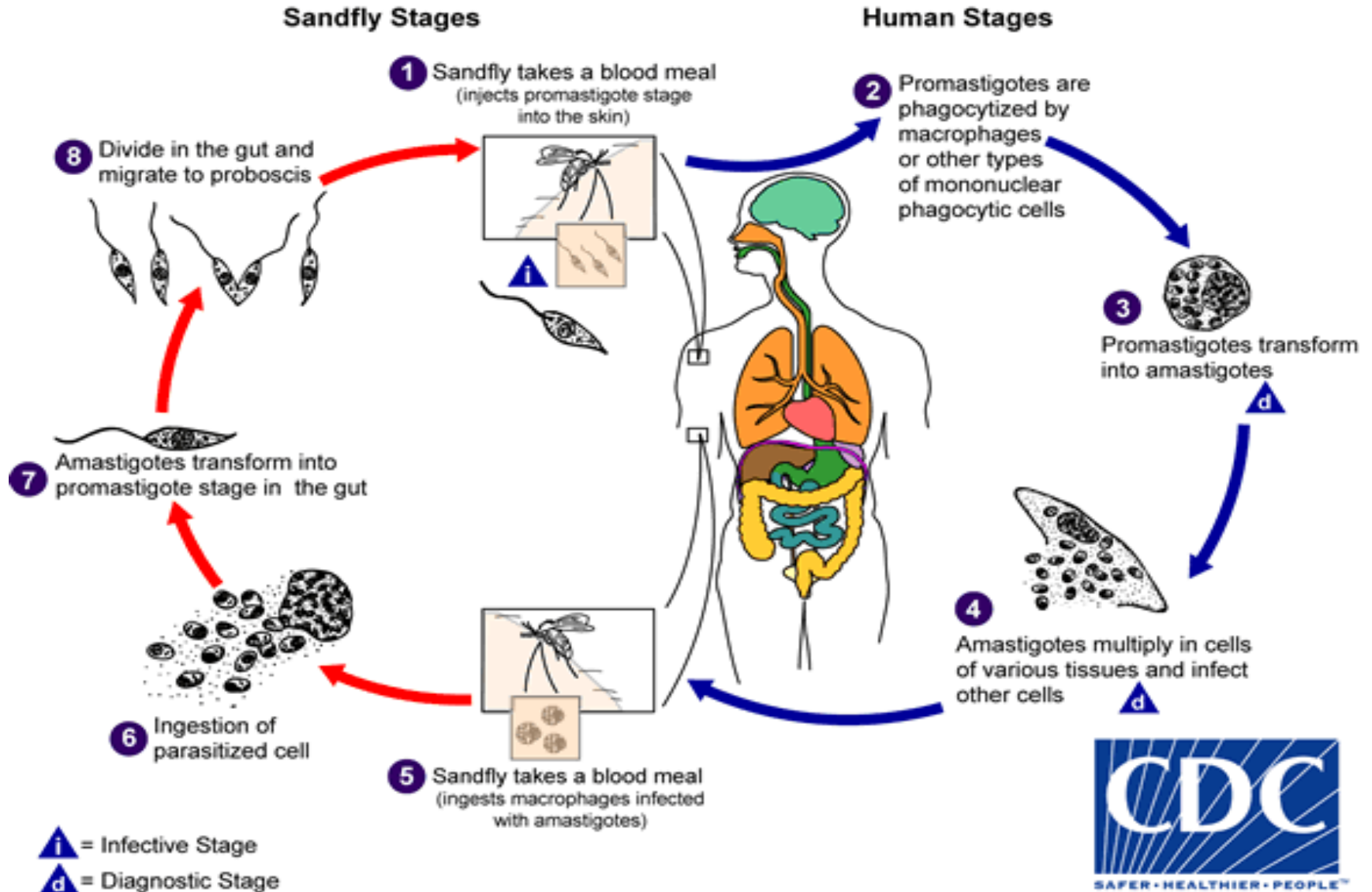
Family Trypanosomatidae

Genus *Leishmania*



- Transmitted to the mammalian hosts by the bite of infected sandflies, *Phlebotomus* and *Lutzomyia*

Leishmania Life cycle



Leishmaniasis

Leishmaniasis is caused by infection with *Leishmania* parasites, which are spread by the bite of phlebotomine sand flies.

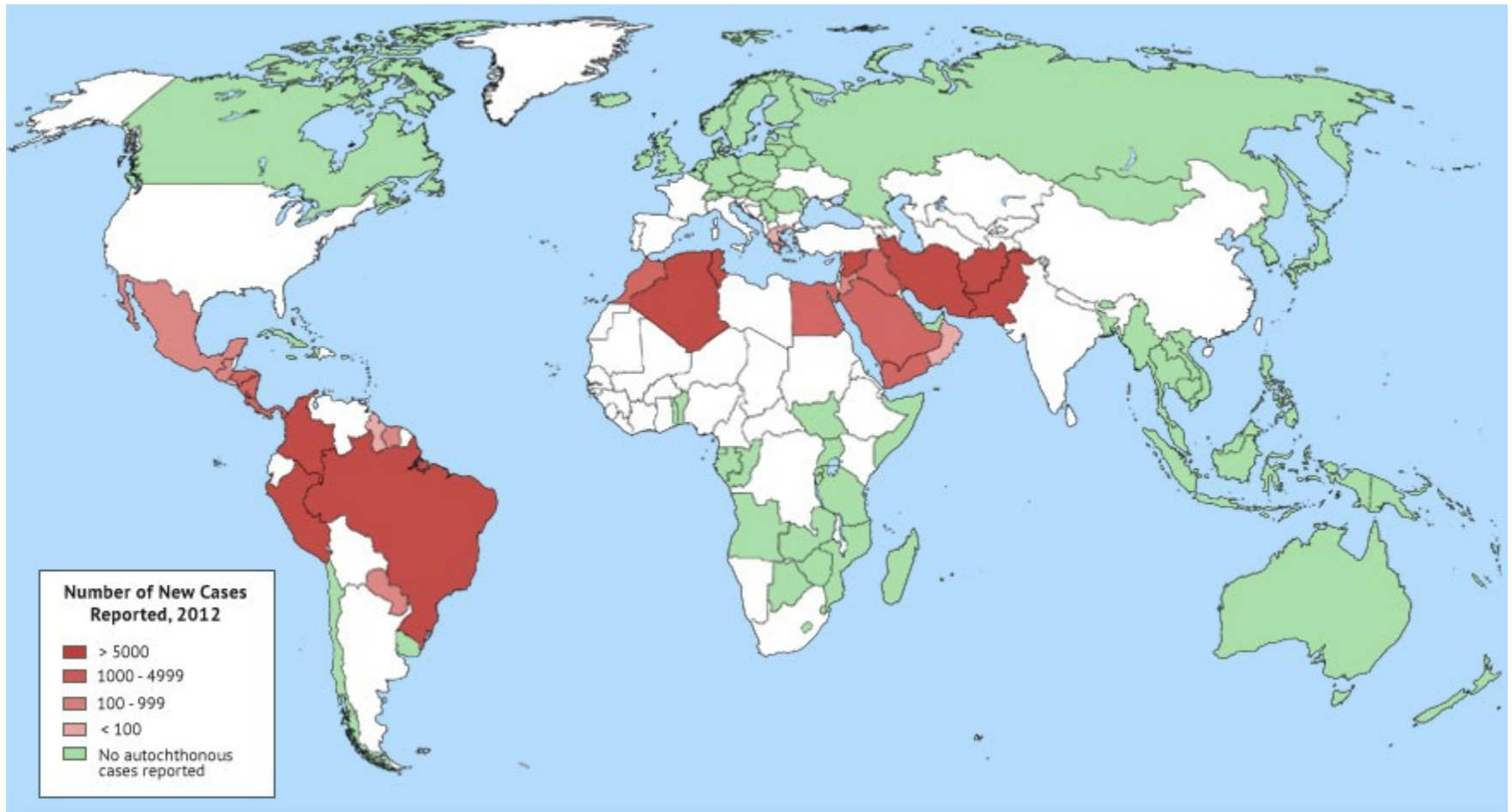
There are several different forms of leishmaniasis in people.

The most common forms are cutaneous leishmaniasis, which causes skin sores, and visceral leishmaniasis, which affects several internal organs (usually spleen, liver, and bone marrow).

Cutaneous Leishmaniasis



Epidemiology of cutaneous Leishmaniasis



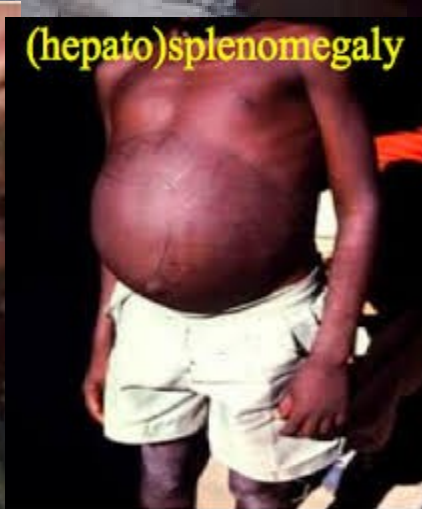
Visceral Leishmaniasis



Slide 082: A typical VL case with malnutrition and anasarca



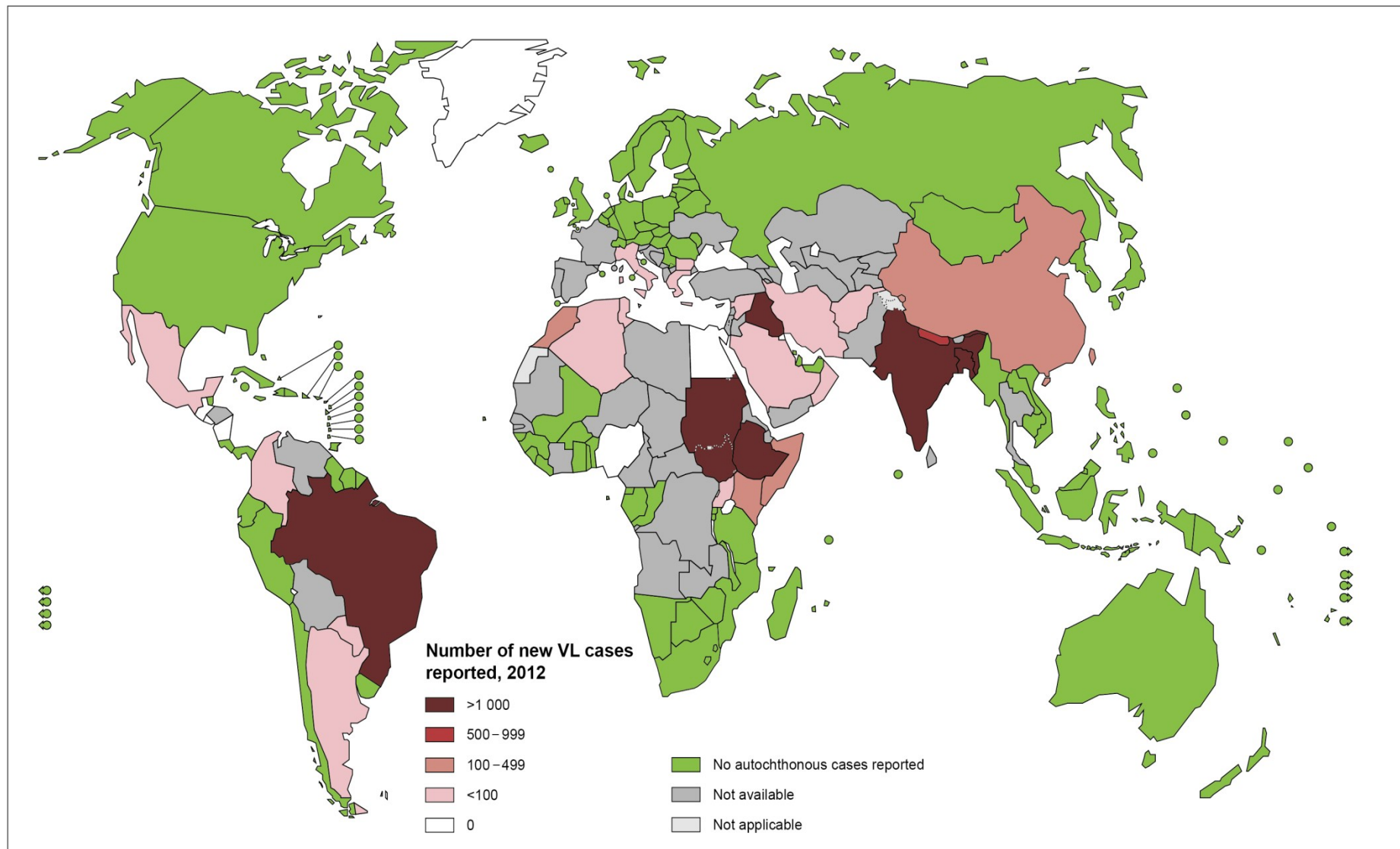
lymphadenopathy



(hepato)splenomegaly



Status of endemicity of visceral leishmaniasis, worldwide, 2012



The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement. © WHO 2013. All rights reserved

Data Source: World Health Organization
Map Production: Control of Neglected
Tropical Diseases (NTD)
World Health Organization

Anti leishmania activities of propolis

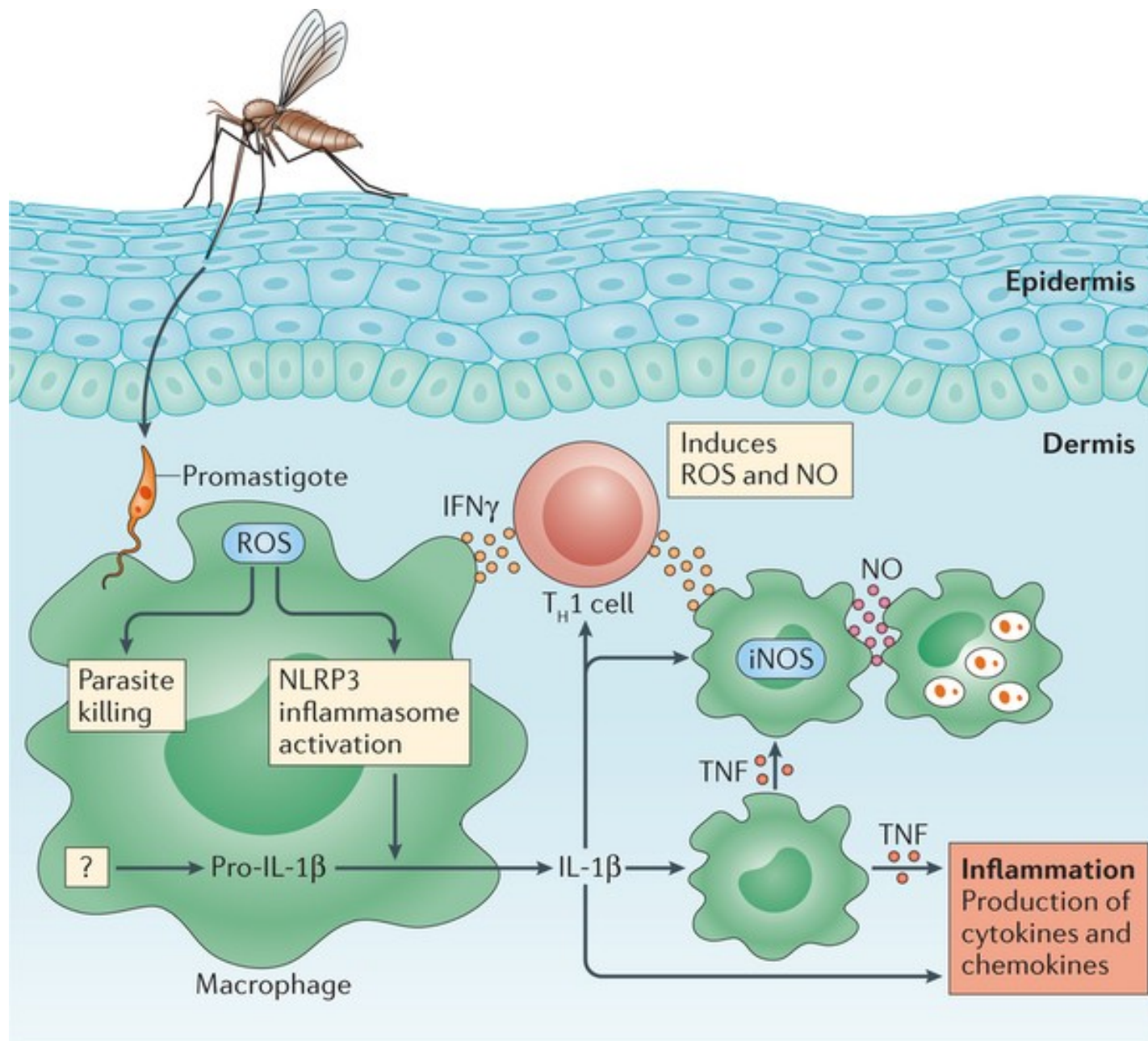
- Effect of four Brazilian propolis ethanolic extracts on *Leishmania amazonensis* performing assays with promastigote forms,
- extracellular amastigotes,
- infected peritoneal macrophages.

Anti leishmania activities of propolis

- reduce parasite load as monitored by the percentage of infected macrophages and the number of intracellular parasites.
- Red propolis contains high concentration of prenylated and benzophenones compounds, was the most active extract against *L. amazonensis*.
- The anti-Leishmania effect of red propolis was increased in a concentration and time dependent manner.
- Red propolis treatment proved to be non-toxic to macrophage cultures.
-

Anti leishmania activities of propolis

- **25 µg/ml Red propolis extract:**
- reduced the parasite load of macrophages
- presented no direct toxic to promastigotes
- presented no direct toxic to extracellular amastigotes
- It was suggested that constituents of propolis intensify the mechanism of macrophage activation leading to killing of *L. amazonensis*.



Human trypanosomiasis

- Human trypanosomiasis affects as many as 66 million people in sub-Saharan Africa. Trypanosomes are also found in the Americas in the form of *Trypanosoma cruzi*, which causes American human trypanosomiasis, or Chagas' disease.
- This disease is found in humans in two forms: as an **amastigote** in the cells, and as a **trypomastigote** in the blood.

Trypanosomiasis

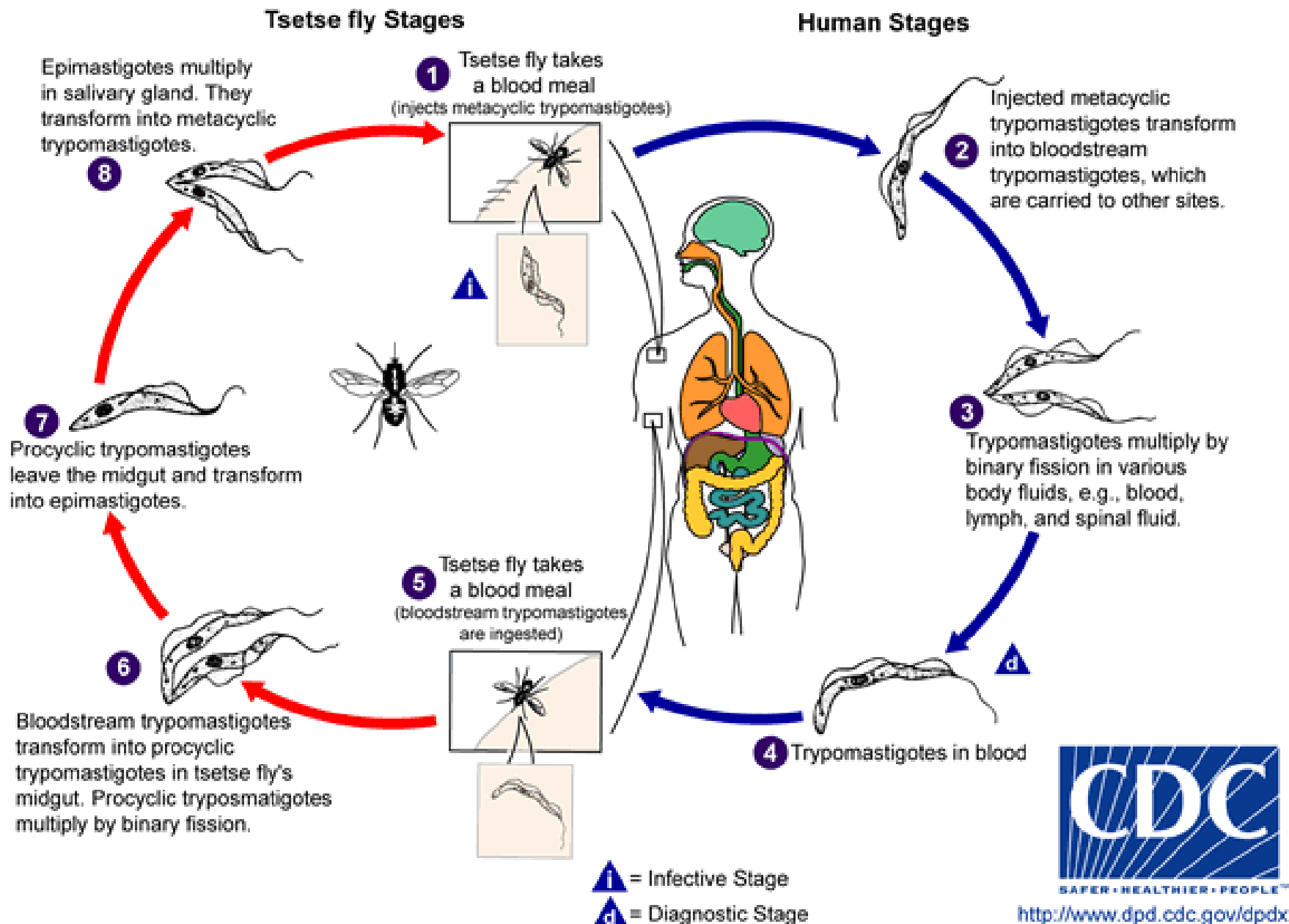
- Trypanosomes infect a variety of hosts and cause various diseases, including the fatal human diseases **sleepin sickness**, caused by Trypanosoma **brucei**, and **Chagas disease**, caused by Trypanosoma **cruzei**

Symptoms of sleeping sickness Disease

**changes of behaviour,
confusion,
sensory disturbances
poor coordination.**

Disturbance of the sleep cycle

Life cycle



Anti trypanosoma activities of propolis

Brazilian green propolis ethanolic extract targets *T. cruzi* and its effect on experimental infection of mice.

The IC_{50} /4 days for inhibition of **amastigote** proliferation was $8.5 \pm 1.8 \mu\text{g mL}^{-1}$, with no damage to the host cells.

In **epimastigotes**, Brazilian green propolis ethanolic extract induced alterations in reservosomes, Golgi complex and mitochondrion.

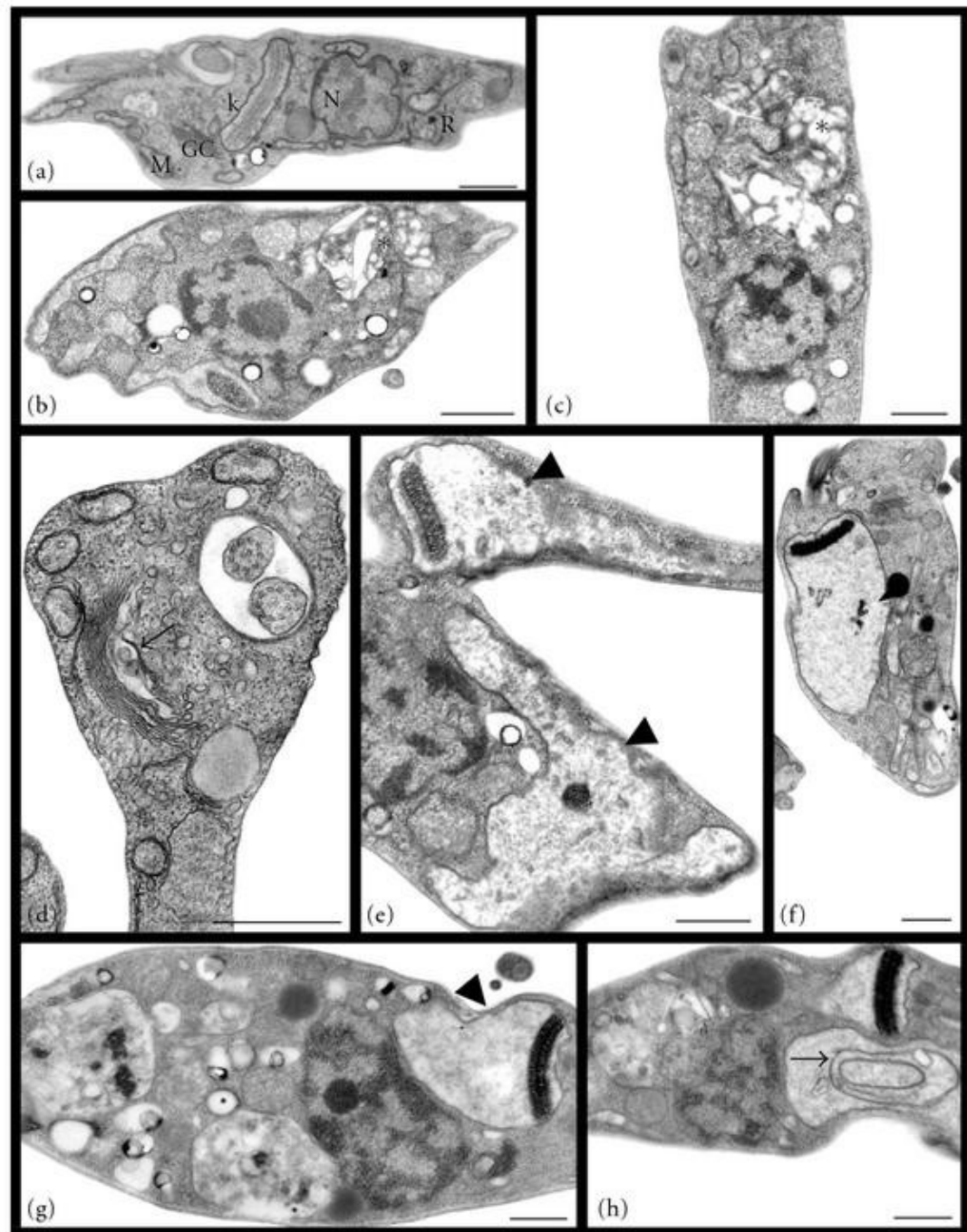
In **trypomastigotes**, Brazilian green propolis ethanolic extract led to the loss of plasma membrane integrity.

The in vitro studies indicate that Brazilian green propolis ethanolic extract interferes in the functionality of the plasma membrane in trypomastigotes and of reservosomes and mitochondrion in epimastigotes.

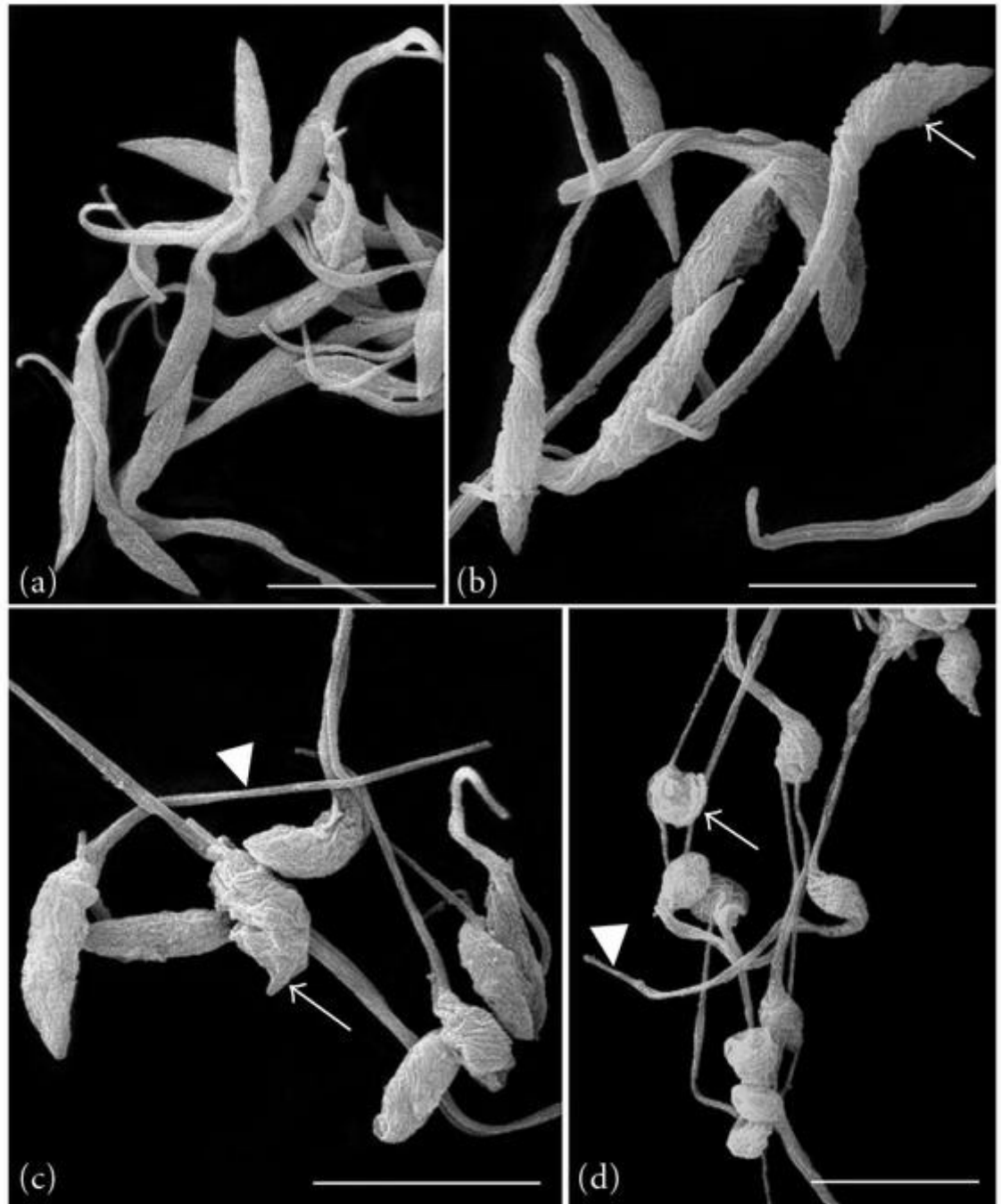
Anti trypanosoma activities of propolis

- Acutely infected mice were treated orally with Brazilian green propolis ethanolic extract and the parasitemia, mortality and GPT, GOT and urea levels were monitored.
- The extract (25–300 mg kg⁻¹ body weight/day for 10 days) reduced the parasitemia, increased the survival of the animals and did not induce any hepatic, muscular lesion or renal toxicity.

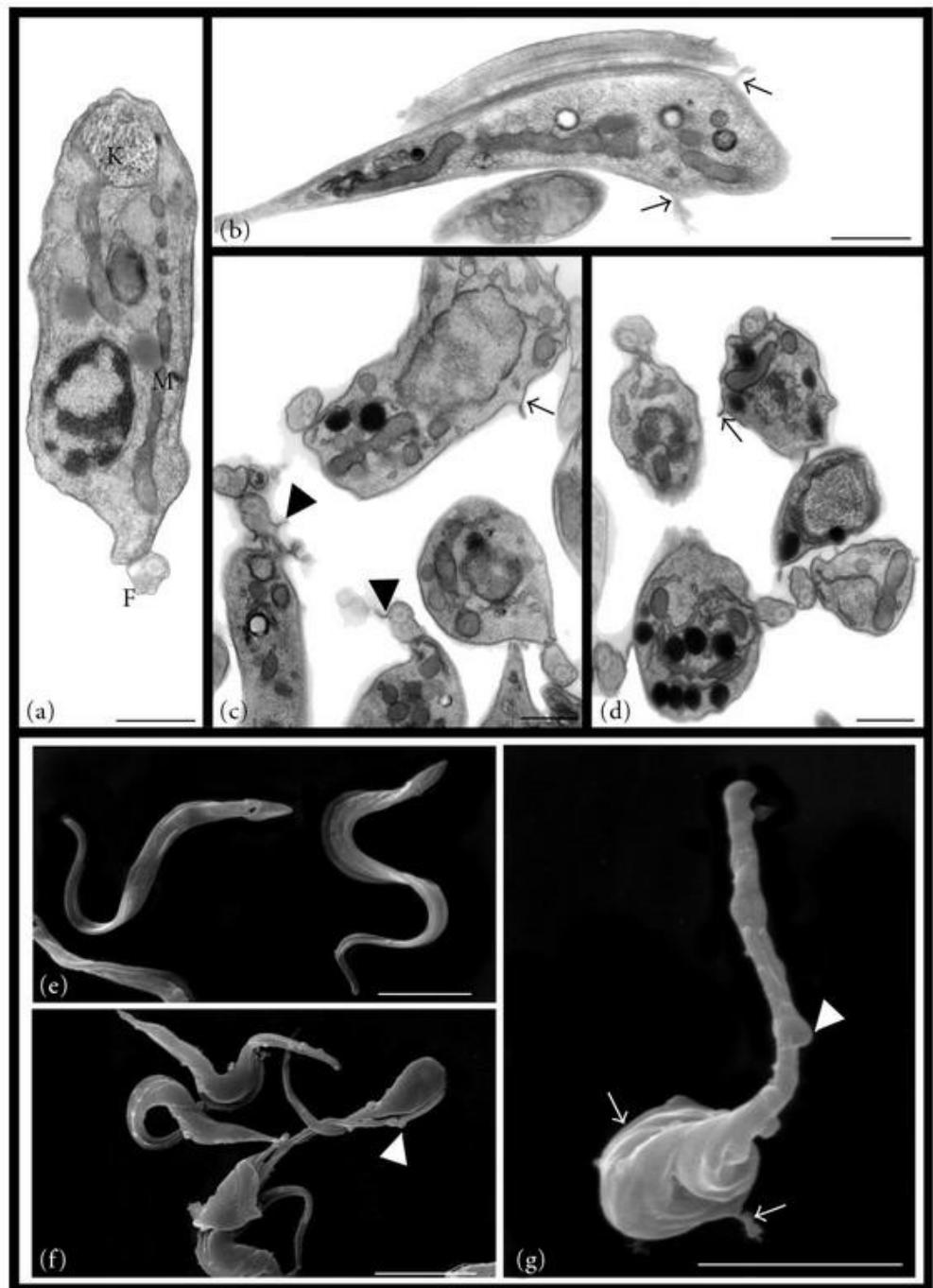
Transmission electron microscopy of *T. cruzi* epimastigotes treated with Et-Bra for 24 h. (a) Control parasite showing the typical elongated body and normal morphology of mitochondrion (M), Golgi complex (GC), nucleus (N) reservosomes (R), and kinetoplast (k); (b) 50 $\mu\text{g mL}^{-1}$ and (c) 100 $\mu\text{g mL}^{-1}$ induced alterations in the morphology of reservosomes (asterisks), with increase of the organelle volume and the number of lipid inclusions and decrease of proteic matrix and vacuolization; (d) 100 $\mu\text{g mL}^{-1}$ induced dilatation of the Golgi complex cisternae (arrow); (e) 100 $\mu\text{g mL}^{-1}$, (f) 250 $\mu\text{g mL}^{-1}$ and (g, h) 300 $\mu\text{g mL}^{-1}$ caused swelling of the mitochondrion with scarcity of the matrix and mitochondrial cristae (arrowheads), with formation of membrane structures inside the organelle (arrow). Bars: 0.5 μm . Anti trypanosoma



Scanning electron microscopy of *T. cruzi* epimastigotes treated with Et-Bra for 24 h: (a) control parasite with normal morphology; (b) 100 $\mu\text{g mL}^{-1}$, (c) 250 $\mu\text{g mL}^{-1}$ and (d) 400 $\mu\text{g mL}^{-1}$ led to gradual alteration on the morphology, from body shortening to rounded parasites (arrow) with preservation of the flagellum (arrowheads). Bars: 4 μm .



Transmission electron microscopy (a–d) and scanning electron microscopy (e–g) of *T. cruzi* trypomastigotes treated with Et-Bra for 24 h: (a) control parasite with the normal morphology of mitochondrion (M), kinetoplast (k) and flagellum (f); (b) 30 $\mu\text{g mL}^{-1}$ and (c, d) 60 $\mu\text{g mL}^{-1}$ induced the formation of blebs on the body (arrow) and flagellar (arrowheads) membranes. Bars: 0.5 μm . (e) control parasite with the normal morphology; (f, g) 60 $\mu\text{g mL}^{-1}$ leading formation of blebs on the body (arrow) and flagellar (arrowheads) membranes and some rounded parasites (small arrow). Bars: 4 μm .



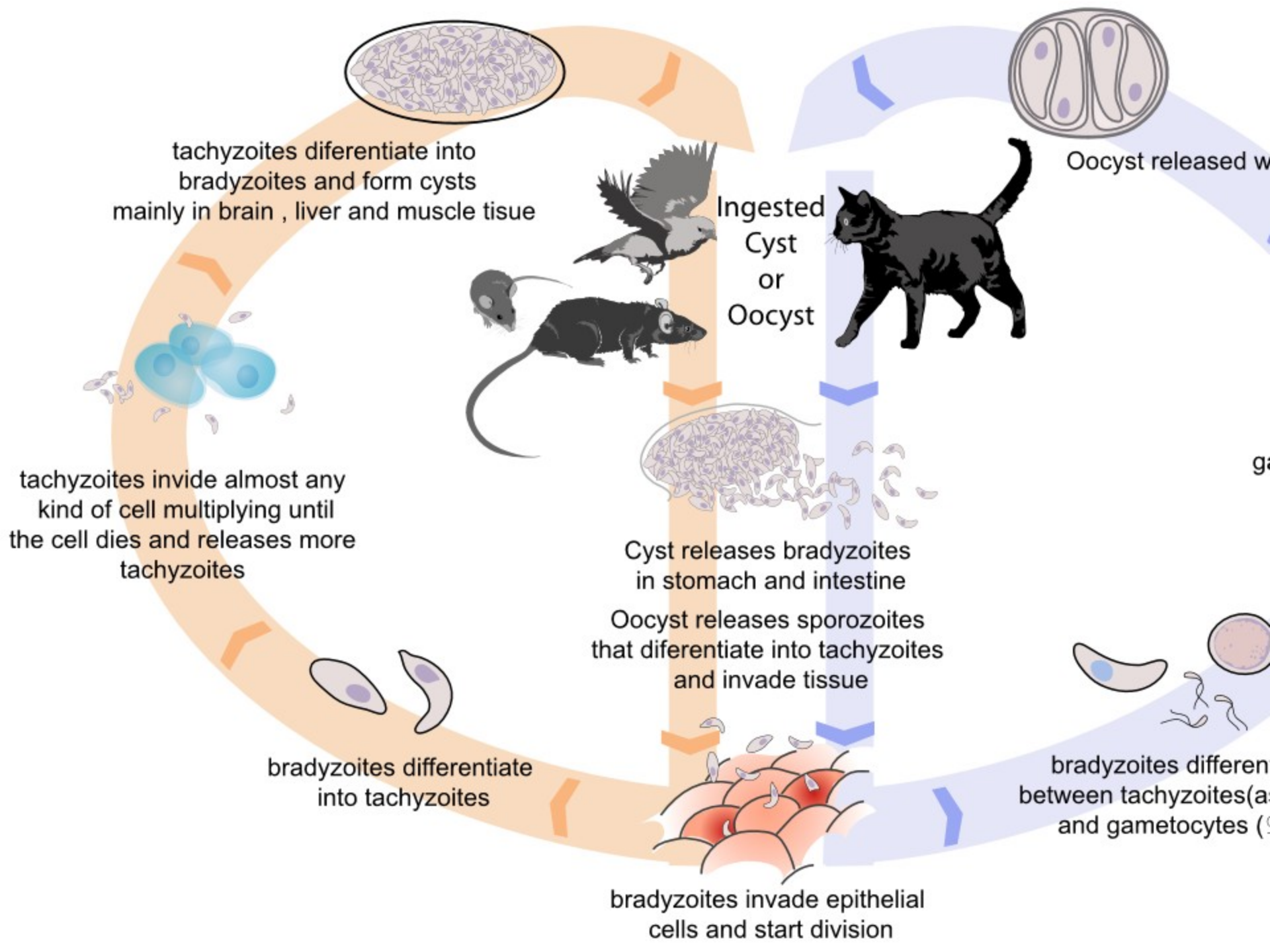
***T. gondii* is capable of infecting virtually all warm blooded animals, but felids such as domestic cats are the only known definitive hosts in which the parasite can undergo sexual reproduction**

In humans, *T. gondii* is one of the most common parasites in developed countries

previous estimates have shown the highest prevalence of persons infected to be in France, at 84%.

Although mild, flu-like symptoms occasionally occur during the first few weeks following exposure, infection with *T. gondii* produces no readily observable symptoms in healthy human adults

This asymptomatic state of infection is referred to as a latent infection and has recently been associated with numerous subtle adverse or pathological behavioral alterations in humans.



Anti toxoplasma

- Antiprotozoal activity is evaluated by an in vitro growth inhibitory effect on a culture of parasites after incubation in the presence of different concentrations of propolis

Concluded remarks

Propolis proved significant anti bacterial, anti viral, antifungal, antitumer and antioxidant activities

Its anti protozoal properties are currently investigated against serious human diseases

In near future, we expect that propolis will be formulated as noval therapeutic drug to control protozoal infections



**Thank you for
attention**