- ***LEISHMANIA*/Leishmaniosis:** morphology, the main species, geographical, distribution, epidemiology, the life cycle, pathogenesis and clinical signs, pathology, diagnosis, treatment and control.
- **TREPOMONADEA (DIPLOMONADIDA: Hexamitidae: *GIARDIA*; *SPIRONUCLEUS*).** Giardiosis and spironucleosis: morphology, prevalence, epidemiology, the life cycle, pathogenesis and clinical signs, pathology, diagnosis, treatment and control.

prof. Alica Kočišová, DVM, Ph.D.

Regnum: **EUKARYOTA, PROTOZOA**

Phylum: **EUGLENOZOA**

Subphylum: **KINETOPLASTA**

Class: **KINETOPLASTEA**

Order: **TRYPANOSOMATIDA**

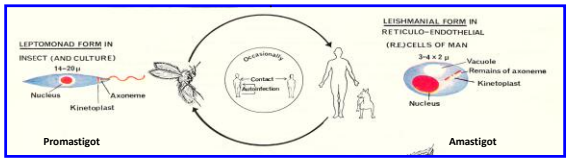
Family: Trypanosomatidae

Genus: *Trypanosoma*
Leishmania

Genus *Leishmania*

- among **50 *Leishmania*** species known to infect humans **13 are zoonotic** **morphologically indistinguishable** and are referred to a **"species complexes"**
- **their differentiation is based on:**
  - biological criteria – clinical picture of diseases, hosts, vectors
  - molecular characteristics

Clinical disease	Old World	New World
VISCERAL	*L. donovani* complex	
	L. infantum complex	*L. infantum* complex
CUTANEOUS	*L. tropica*	
	L. major	
	L. aethiopica	
MUCOCUTANEOUS		*L. mexicana* complex
		L. brasiliensis complex



CYCLICAL TRANSMISSION – arthropod vector

Vectors

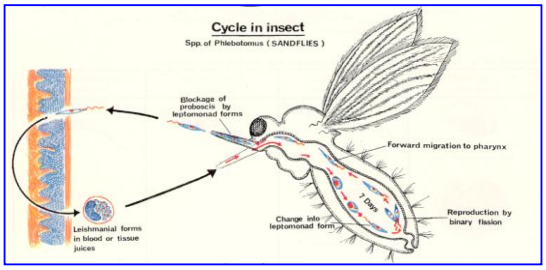
***Lutzomyia* spp.**

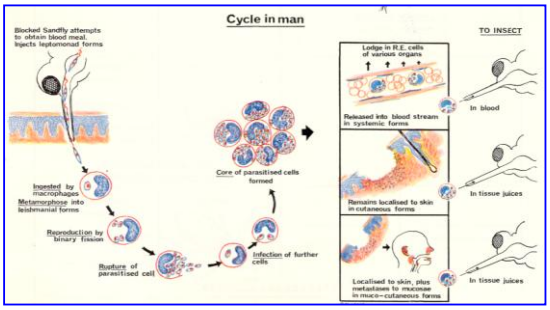
***Phlebotomus* spp.**

Human Leishmaniosis

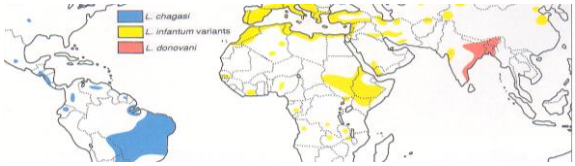
Sir William Boog Leishman (1865-1926), Scottish pathologist and British Army medical officer;
Detected the causative agent of leishmaniosis in 1901 in small child (infant)

***Leishmania infantum* – life cycle**





Leishmania spp./visceral leshmaniosis – geographical distribution



HUMAN LEISHMANIOSIS
- every year 1,5 - 2 mil. new cases occur
- 320 – 350 mil. people are in risk
- 12 mil. are already infected

Seroprevalence of *L. infantum* in dogs in endemic **Mediterranean regions**: 1 – 50 %
Reservoir hosts: foxes, cats, wild rabbits, hares, rats ...

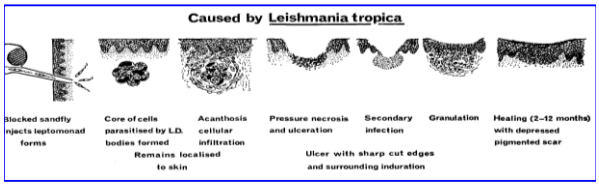
Visceral Leishmaniasis

***L. donovani* complex**
***L. infantum* complex**

- Most severe form of the disease, may be fatal if left untreated;
- Usually associated with **fever, weight loss**, and an **enlarged spleen and liver;**
- Anemia (low RBC), leukopenia (low WBC), and thrombocytopenia (low platelets) are common;
- **Lymphadenopathy** may be present;
- Visceral disease from the Middle East is usually milder with less specific findings than visceral leishmaniasis from other areas of the world
- Symptoms usually **occur months after sandfly bite**
  - Soldiers from Desert Storm presented up to five months after leaving the Persian Gulf;
  - Because symptoms are non-specific and often start after redeployment there is usually a delay in diagnosis;
  - Visceral leishmaniasis should be considered in any chronic FEVER patient returning from an endemic area.

Cutaneus leishmaniosis

L. tropica
L. major
L. aethiopica



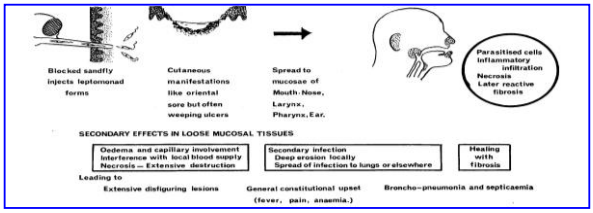
Cutaneous Leishmaniasis

Most common form:
- Characterized by one or more **sores**, **papules** or **nodules** on the skin;
- Sores **can change in size** and appearance over time;
- Often described as looking somewhat like a volcano with a raised edge and central crater;
- Sores are **usually painless** but can become **painful if secondarily infected;**
- Swollen lymph nodes may be present near the sores (under the arm if the sores are on the arm or hand...);
- Most sores develop within a few weeks of the sandfly bite, however they can appear up to months later;
- Skin sores of cutaneous leishmaniasis can heal on their own, but this can take months or even years;
- Sores can leave significant **scars** and be disfiguring if they occur on the face;
- If infection is from ***L. tropica*** it **can spread to contiguous mucous membranes** (upper lip to nose)

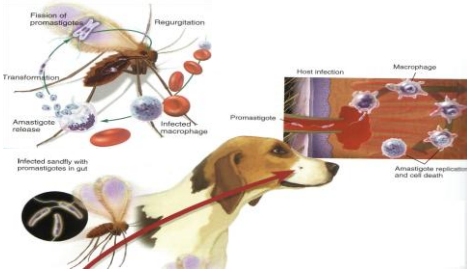
Muco-cutaneus leishmaniosis (Espundia)

L. mexicana complex

L. brasiliensis complex



Leishmaniosis in dogs



Leishmaniosis – clinical symptoms in dogs

In endemic areas >50% of dogs may be infected without clinical signs. Clinical leishmaniosis starts as a **visceral form**, cca 90% of the dogs present also **cutaneous lesions**

VISCERAL FORM - chronic systemic disease

- **incubation period:** 1 – 3 months to several years
- generalized **lymphadenopathy**
- **weight loss, anorexia, apathy**
- **hepato- and splenomegalia**
- **hypoalbuminaemia**
- **renal failure with proteinuria** increased urination and drinking

CUTANEOUS FORM

- **skin lesions** (starting in the head)
  - alopecia, branlike desquamation
  - nodules, pustules, excessive growth of claws

IT IS POSSIBLE TO SEPARATE THE DOGS IN TWO POPULATIONS:

➢ susceptibles

- activation of **Th2 cells**
- high production **non-specific Ab**
- lack of cell-mediated immunity

genetic factors

➢ resistants

- activation of **Th1 cells**
- low level of **specific Ab**
- efficient cell-mediated response

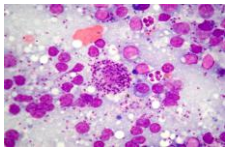
factors related to parasite

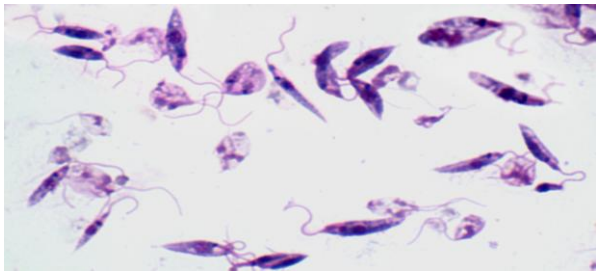
Diagnosis of leishmaniosis

DIRECT CONFIRMATION
finding of **amastigotes** in smears from
- skin scraping of ulcers
- biopsy from lymph nodes
- splenic aspirates, bone marrow

SEROLOGICAL METHODS
- IFAT
- ELISA
- Western blotting
- direct agglutination assays

PCR METHODS





promastigotes following in vitro culture

Leishmaniosis-HIV coinfection

latent infection that reactivated when the immune system is attached by the virus or…..

……new acquired infection in people with a low number of lymphocytes

Prevention and control of leishmaniosis

- Most cases of CL heal without treatment
  – immune to further infection
- Other forms of leishmaniosis are **extremely difficult to treat**, often **requiring a long course of disease** – **often relapses**

DRUG	DOSE (mg/kg)	ROUTE	INTERVAL (hrs)	DURATION (weeks)
miltefosine **Milteforan** (in Europe)	2	per os	24	4
pentavalent antimony drugs (meglumine antimonate) **Glucantime, Merial**	100	i.v. s.c.	24	3 – 4
sodium stibogluconate **Pentostam**	30 – 50	i.v. s.c.	24	3 – 4
Allopurinol (off label)	20	per os	12 – 24	months - years

Medical treatment of infected dogs is ineffective in eliminating the parasite

Antimonials + Allopurinol

Problems related to the therapy in dog

- long terms
- negative side effects
- cost
- parasitological cure
- relapses

Control in endemic areas

today the eradication of canine leishmaniosis

is not reachable .....

... but control measures can reduce the risk of infection in dogs in endemic areas and consequently in humans

Prevention and control of leishmaniosis

- Avoid of sandfly bites through use of repellents or insecticides (permethrin, pyroproxyfene, deltamethrin – Scalibor) – spot on, collar, spray, solution;
- Vector control, based on spraying with residual insecticides, can be effective inside and around kennels;
- Killing symptomatic and seropositive dogs – nonsymptomatic dogs and wild canids are source of infection;
- Vaccination of dogs against VL (attenuated or killed whole parasites, recombinant antigens, DNA vaccines);

Control - vaccines

- first generation vaccines (killed whole Leishmania parasites) tested in Iran, Sudan, Latin America
- second generation vaccines (recombinant antigens) several antigens offer promise as candidate
- CaniLeish

DIPLOMONADIDA

***GIARDIA*/GIARDIOSIS** and ***SPIRONUCLEUS*/SPIRONUCLEOSIS**.
Characteristic, classification, morphology, location, prevalence, epidemiology, the life cycle, pathogenesis and clinical signs, pathology, diagnosis, treatment and control.

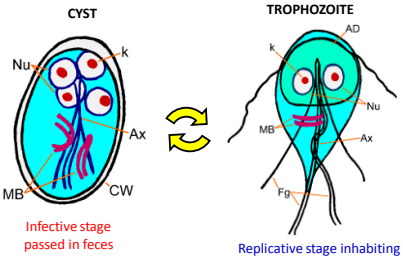
Regnum: **EUKARYOTA, PROTOZOA**
Phylum: **METAMONADA**
Class: **TREPOMONADEA**
⇒ Order: **DIPLOMONADIDA**
⇓
Family: Hexamitidae
⇓
Genus: ***Giardia*** ***Spironucleus***

⇒ Order: ENTEROMONADIDA
⇒ Order: RETORTAMONADIDA

Giardia /Giardiosis

- bilaterally symmetrical with duplicate organelles including nuclei

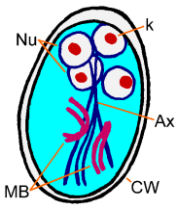
Giardiasis - characterized by catarrhal gastroenteritis, especially young and debilitated individuals. **Zoonoses.**
Belongs to **opportunistic infections.**



Infective stage passed in feces

Replicative stage inhabiting small intestine

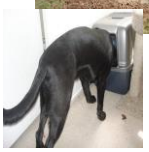
Key Features of Cysts



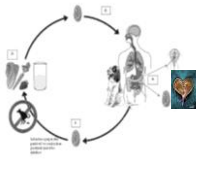
- **oval shape**
- 11-14 x 6-10 µm
- distinct cell wall set apart from cytoplasm
- **4 nuclei at anterior end**
  - large karyosome, no peripheral chromatin
- fibrils (axonemes) evident
- median bodies

TRANSMISSION

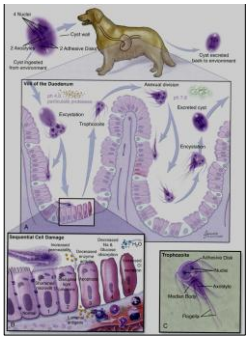




Giardia duodenalis LC



1. Oral uptake of cysts after faecal contamination of food or water
2. Trophozoites excyst in small intestine and may divide by binary fission
3. Trophozoites are attached to the surface of intestinal villi
4. free trophozoites encyst in the intestine and passed in faeces



Assemblages (genotypes)

G. duodenalis is divided into eight genetically different assemblages (A–H)

ASSEMBLAGE (GENOTYPE)	SPECIES	HOST
A	*G. duodenalis*	humans, and other primates, dogs, cats, livestock, rodents, wild mammals
B	*G. enterica*	Humans and other primates, dogs, chinchilla, beaver, wild animals
C/D	*G. canis*	Dogs, cats, coyotes, wolves, other canids
E	*G. bovis*	Cattle, hoofed livestock
F	*G. cati*	cats
G	*G. simondi*	rats
H		seal

Pathogenesis

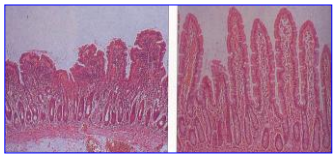
- epithelial damage
  - villus athrophy
  - crypt cell hypertrophy
  - cellular infiltration
  - inflammation of mucosa
- malabsorption
- enzyme deficiencies
  - lactase (lactose intolerance)

Possible Mechanisms

- mechanical irritation
- obstruction of absorption

Pathogenesis

- Infections cause severe intestinal disorders, diarrhea;
- Symptoms include abdominal distension, nausea, flatulence, and weight loss;
- Attachment of the trophozoite to the mucosal surface by means of the adhesive disc causes shortening of the vili of the small intestine;
- Inflammation of the crypts and lamina propria, and lesions on mucosal cells;



Pathogenesis

- **Severe infections produce malabsorption syndrome – inability of the small intestine to absorb essential fat – soluable**
- **There may also be a reduction in the secretion of a number of small intestinal digestive enzymes**

Clinical signs:

The spectrum varies from asymptomatic course to severe diarrhea and malabsorption.

Acute giardiosis develops after an incubation period of 5 to 6 days and usually lasts 1 to 3 weeks.
Symptoms: diarrhea, abdominal pain, bloating, nausea, and vomiting.

Chronic giardiosis the symptoms are recurrent and malabsorption and debilitation may occur.

Giardia duodenalis – Clinical signs

Giardia infects the cells of the small intestine blocking nutrient absorption.

If the infection is clinically manifested, gastrointestinal symptoms frequently included **diarrhoea (watery, steatorrhea, pale, foul smelling)**, often alternating with constipation, a **spastic pain** in the epigastric region, **flatulence;**

Soft diarrhea containing: mucus, bad smell, greasy texture
- Vomiting
- Apparent weight loss
- Lethargy

DIAGNOSIS

Centrifugal Fecal Flotation

– Zinc sulphate (FAUST SOLUTION)

– Scan microscope slide at 40X

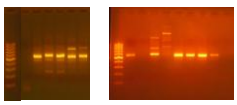
– Cysts

- **Intermittent shedding**
- **3 fecal exams in 5-7days**

DIAGNOSIS

1. **Flotation method - for the presence of *Giardia* cysts**

2. The nested PCR - using specific primers to amplify a sequence of the triose phosphate isomerase ***(tpi),*** *glutamate dehydrogenase* ***(gdh),*** *β-giardin* ***(bg)*** genes.



THERAPY

- For dogs and cats: **fenbendazole** or **febantel** (Drontal plus tbl., Panacur Pet; ...) - **repeated application required**; for **at least 3 days**;
- **metronidazol** (Metrobactin 500 tbl.)

A post-treatment faeces examination is essential

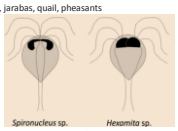
PREVENTION
- adherence to zoohygienic measures;
- cysts resistant, survive in the months of the environment; flies - mechanical, passive vector!
- nutrition - protein suppresses giardiasis; carbohydrate supports;

PROGNOSIS
- very good
- in immunosuppression - dubious

SPIRONUCLEUS (*Hexamita*)

Spironucleosis is characterized by **duodenitis, enteritis.**

Hexamita (Spironucleus) meleagridis - turkeys, pheasants, jarabas, quail, pheasants
Hexamita columbae – pigeons
Hexamita muris - mice, rats, voles, hornet
Hexamita salmonis - fish
Hexamita truttae - fish



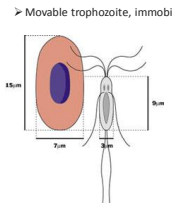
- trophozoites binucleate, with 8 flagella;
- ventral adhesive disk absent
- trophozoites undergo binary fission in intestine;
- cysts passed in feces

localization
- Lieberkühn crypts of small intestine, caecum, Fabrici stock, liver, abdominal cavity

reproduction
- **longitudinal** division
- forms oval **8-nuclei cysts (4-5x6-7 µm)**

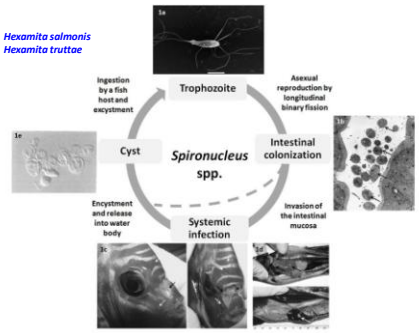
Spironucleus (*Hexamita*) *meleagridis*

- Movable trophozoite, immobile cyst



Size compared to Ec

Location in GIT of turkey



- Incubation 1 week
- Disease of young animals (turkeys at 10 weeks of age); high mortality (80%)
- outgoing feathers, dehydration, diarrhoea (watery), weight loss
- Pathology includes diarrhoea, weight loss, death

- Find trophozoites and cysts in droplets of intestinal contents after staining, for example, haematoxylin
- Autopsy – catarrhal inflammation of the small intestine, with a lot of spilled fluid

- Treatment not known (**nitro imidazole** derivate - PROHIBITED)

- Prevention - hygiene, nutrition, separate breeding of young birds (turkeys)


THANKS!