Parasites of Laboratory Mice and Rats and Their Effects on Research

Bogdan Băcescu, Spiru Haret University, Bucharest

ARSAL - BUCHAREST, 2016
Why are we talking about parasitic diseases on laboratory mice and rats?

Parasitic diseases they develop slowly, often without obvious clinical expression to attract attention, although the presence of the parasite involves a detectable reaction of the parasitized body and who can leave their mark on physiological values of the animal used in the experiment.
When are we talking about parasitic diseases laboratory mice and rats?

Current systems increase limits contact with parasitic contamination sources but we can discuss about the introduction of specimens contaminated with asymptomatic or technical mistakes that allow contamination of feed, bedding or water.

Exclusive use of lines in experiments that reduce the reactivity of the body and enable the emergence of systemic parasitosis: toxoplasmosis, microsporidiasis, cryptosporidiosis, leishmanioses.

Absence of any preventive examination which can be detected parasitic forms.
**Phylum Apicomplexa**

*Class Coccidia*
- Cryptosporidium muris
- Cryptosporidium parvum
  - Eimeria falciformis
  - Eimeria miyairii
  - Eimeria nieschulzi
  - Eimeria praegensis
  - Eimeria separata
  - Frenkelia sp.
- Hammondia hammondi
- Hepatozoon muris
- Hepatozoon musculi
  - Isospora ratti
- Klossiella muris
- Sarcocystis muris
- Toxoplasma gondii

*Class Piroplasmidia*
- Babesia microti
- Babesia rodhaini

*Class Haemosporidia*
- Plasmodium inopinatum

**Phylum Microsporidia**
- Encephalitozoon cuniculi
TOXOPLASMOSIS

Systemic parasitic disease

- contamination is usually by oral ingestion of oocysts from cat feces;
- rodent contamination is possible by cannibalism (infected bodies)
- other sources of contamination; bedding or water with uncertain origin
- It has long evolution, asymptomatic common in immunocompromised animals
- from the intestinal gut trophozoites are vehicle by CD11c and CD11b in systemic circulation and they spread in different tissues and organs where are pseudocysts and cysts
- Brain cysts can resist many times without clinical signs, but in experiments that use of immunosuppression based on dexamethasone they may be activated (mice model recrudescence on Toxoplasma).

Murine Model of Drug-induced Reactivation of Toxoplasma gondii- Olgica Djurkovic Djakovic and vladimir Milenkovic Toxoplasmosis Research Laboratory, Institute for Medical Research, Belgrade, Yugoslavia

Toxoplasma can develop on rat and mice comportamental disorders- neophilia, self exposure on carnivores predators, cat urine smell attraction considered like “schizophrenic deviation”

Behavioral changes induced by Toxoplasma infection of rodents are highly specific to aversion of cat odors Ajai Vyas *, †, Seon-Kyeong Kim †, Nicholas Giacomini vol. 104 no. 15 6442–6447, doi:10.1073/pnas.0608310104

TOXOPLASMOSIS
compared the effects of mouse infection by two Type II strains of *T. gondii*, Prugniaud (PRU) and ME49, on attraction to cat odour, locomotor activity, anxiety, sensorimotor gating, and spatial working and recognition memory 2 months post-infection (mpi). Attraction to cat odour was reassessed 7 mpi. At 2 mpi, mice infected with either strain exhibited significantly more attraction to cat odour than uninfected animals did, but only PRU-infected mice exhibited this behaviour 7 mpi. PRU-infected mice had significantly greater body weights and hyperactivity, while ME49-infected mice exhibited impaired spatial working memory. No differences in parasite antibody titres were seen between PRU- and ME49-infected mice. The present data suggest the effect of *T. gondii* infection on mouse behaviour is parasite strain-dependent.

Neospora caninum is an obligate intracellular parasite that causes neurological disorders in dogs and cattle. The majority of host animals are asymptomatic at the chronic stage of infection. However, it remains unclear whether cerebral function is normal in asymptomatic animals.

In the brains of infected mice, parasites were distributed throughout the brain and histological lesions were observed everywhere except for the cerebellum. Expression levels of proinflammatory cytokines, interferon-gamma and tumour necrosis factor-alpha, were highly upregulated in several brain regions of infected mice.

Interestingly, the expression levels of immediately early genes, c-Fos and Arc, in the brain of infected mice were lower than those of in uninfected mice. Our findings may provide insight into neurological disorders associated with N. caninum infection.

Ihara F¹, Nishimura M¹, Muroi Y², Furuoka H², Yokoyama N¹, Nishikawa Y¹.- Changes in neurotransmitter levels and expression of immediate early genes in brain of mice infected with Neospora caninum.
**Klossiella muris**

- *Klossiella muris* is a one-host parasite in the family *Klossiellidae*, and is the type species for the genus. The sporocyst is the antemortem diagnostic stage, has a thin wall, measures 16 μ by 13 μ, and contains 30 to 35 banana-shaped sporozoites.

Historically, prevalence was high in laboratory colonies but low in wild populations. Current prevalence in wild and laboratory rodents is unknown.

- The mouse is infected by ingesting sporulated sporocysts. Sporozoites are released and distributed hematogenously to the endothelial cells of the renal glomeruli, where schizogony occurs.
- Merozoites enter the epithelial cells of the convoluted tubules of the kidney, where they form gamonts which become macrogametes and microgametets. After fertilization, the zygote or sporont grows and divides by budding to form 12 to 16 sporoblasts, each of which becomes a sporocyst.
- The sporocysts rupture the host cell and pass out of the body in the urine.

**Pathologic Effects.** *Klossiella muris* is generally considered nonpathogenic, but may result in interstitial nephritis. Kidneys may be enlarged, and pale areas and small gray necrotic foci are seen on the surface.
- Microscopically, organisms occur in the tubular epithelial cells glomerular endothelium, and convoluted tubule lumens.
- The parasites are most common in the cortex, where they cause little inflammation, although destruction of tubular epithelium has been reported. Foci of interstitial pneumonia, pulmonary congestion, and splenomegaly are additional lesions which may be observed in animals infected with *K. muris*.

Klossiella muris in mouse kidney. (A) Schizogony. (B), (C) Early stage of sporogony. (D) Late stage of sporogony. Courtesy of N. Meshorer, Weizmann Institute of Science
CRYPTOSPORIDIASIS

- **Morphology.** *Cryptosporidium* sp. belongs to the family Cryptosporidiidae. Mice may be infected by two species of Cryptosporidium: *C. muris* and *C. parvum*. Oocysts of *C. muris* measure 5.3 μ by 7.9 μ.

- **Hosts.** *Cryptosporidium muris* or *C. muris*-like coccidia have been reported from a wide range of mammalian hosts. Recent morphologic and phylogenetic analyses have demonstrated that *C. muris* is not a uniform species, and that the isolates primarily infecting rodents probably constitute a different species from that infecting cattle and other large mammals.

- Gastric glands of naturally infected mice become filled with numerous free or embedded parasites. The glands contain degenerated and atrophied epithelial cells, but there is no evidence of inflammation. Other investigators have reported that infected mice develop gastric mastocytosis, which peaks on days 20 to 30 post-inoculation. Experimentally infected nude mice develop dilated, hypertrophied gastric glands which are filled with numerous parasites. Resistance to disease caused by *C. muris* is age-related, and develops quickly after weaning. Susceptibility has also been linked to differences in major histocompatibility complex genes.

- These differences are correlated with differences in patterns of interleukin-4 secretion.

- Immunocompetent hosts show no clinical signs of infection. In contrast, in experimentally infected neonatal, severe combined immunodeficient (SCID), and other immunodeficient or immunosuppressed mice or rats, a severe and eventually fatal chronic infection occurs, characterized by diarrhea, dehydration, and depression, with infection in the ileum, cecum, and colon.


MICROSPORIDIOSIS

Encephalitozoon cuniculi infects several species of mammals, including rats, mice, and humans.

The extent to which strains are cross-infective and/or represent distinct species is unknown. Though never common in laboratory rodent colonies, E. cuniculi is now considered rare.

In rodents, infection with E. cuniculi has been primarily associated with lesions in the brain, kidneys, and to a lesser extent, liver. In one study, granulomatous encephalitis caused by E. cuniculi was found throughout the brain in 21% of 365 adult laboratory rats. The granulomas (glial nodules) consisted of collections of activated glial cells surrounded by lymphocytes.

Perivascular spread of sporoblasts was observed in the brain, without an associated inflammatory response. The pathogen was also found in interstitial infiltrates in rat kidneys.

Historically, E. cuniculi was a frequent contaminant in cell cultures. Several years ago, Petri reported the infection in 25% of tumor cells of a transplantable ascites sarcoma. The infected tumor cells became less pathogenic than usual and did not give rise to solid tumors after subcutaneous inoculation of rats. Rats and mice infected with E. cuniculi typically show no clinical signs.


Phylum Sarcomastigophora

Class Mastigophora (flagellates)

Hemoflagellates
Trypanosoma conorhini
Trypanosoma lewisi
Trypanosoma musculi
Chilomastix bettencourtii

Giardia muris
Giardia simoni
Hexamastix muris
Octomitus pulcher

Pentarichomonas hominis
Retortamonas sp.
Spironucleus muris
Tetraichomonas microti
Trichomitus wenyoni
Tritrichomonas minuta
Tritrichomonas muris

Endolimax ratti
Entamoeba muris
• Spironucleus muris infects the mouse, rat, hamster, and various wild rodents throughout the world. Historically, S. muris was commonly found in laboratory colonies of rats and mice. Current prevalence is unknown but probably still high.

Inbred mouse strains differing in major histocompatibility type also differ in magnitude and course of infection. For example, 129/J mice experimentally infected with S. muris shed significantly fewer cysts than BALB/c, ByJ, C3H/HeJ, and DBA/1J mice. Immune-deficient animals, such as athymic (nu/nu) or irradiated rats and mice, develop severe chronic enteritis and weight loss following infection with S. muris. Intestinal crypts are hyperplastic and may be distended with trophozoites. Microvilli and villi may be shortened, and enterocyte turnover is increased. There is an increased number of intraepithelial lymphocytes from three weeks onward corresponding to the start of elimination of the infection.

• Courtesy of A. Meshorer, Weizmann Institute of Science.

CESTODES

Cataenotaenia pusilla
Hymenolepis diminuta
Rodentolepis nana
Rodentolepis microstoma
Rodentolepis straminea
Taenia taeniaformis

- Superfamily Rhabditoidea
  - Strongyloides ratti
  - Strongyloides venezuelensis
- Superfamily Heterakoidea
  - Heterakis spumosa
- Superfamily Oxyuroidea
  - Aspiculuris tetraptera
  - Syphacia muris
  - Syphacia obvelata
- Superfamily Trichostrongyloidea
  - Heligmosomoides polygyrus
  - Nippostrongylus brasiliensis
- Superfamily Metastrongyloidea
  - Angiostrongylus cantonensis
  - Angiostrongylus costaricensis
- Superfamily Filaroidea
  - Litomosoides carinii
- Superfamily Trichuroidea
  - Calodium hepaticum
  - Capillaria spp.
  - Trichuris arvicola
  - Trichuris muris
  - Trichosomoides crassicauda
• *Aspiculuris tetraptera* remains common in laboratory mice. Other rodents are also susceptible to infection - *Apodemus, Clethrionomys, Cricetus, Mastomys, Meriones, Microtus, Peromyscus, Rattus*. The eggs are resistant to dessication and many disinfectants, but are sensitive to high temperatures. Infection is by ingestion of infective eggs. Larvae hatch and develop in the posterior colon and then migrate anteriorly and develop to maturity in the proximal colon. They remain in the lumen of the intestine and do not invade the mucosa.

• *Syphacia muris* primarily infects rats, including non-domestic. Other susceptible hosts include laboratory mice, gerbils, and the Syrian hamster. Infections in laboratory colonies remain common. Like other pinworm infections of rodents, *S. muris* is generally considered to be nonpathogenic. Rats infected with *S. muris* have impaired intestinal electrolyte transport and decreased weight gain.
• *Syphacia obvelata*, the common mouse pinworm, is one of the most common parasites of laboratory mice. Many laboratory mouse colonies were infected until recently. Advances in husbandry, and facility design and attention to pathogen detection and control have greatly reduced the prevalence of infection, though it remains high. Other susceptible species include hamsters, gerbils, voles, *mastomys* (*Praomys coucha*), Algerian mice (*M. spretus*), and primates.

• No specific enteric lesions have been attributed to light infections with *S. obvelata*. In contrast, very heavy parasite loads may lead to catarrhal enteritis, hepatic granulomas, and perianal irritation. In spite of their seemingly innocuous nature, infection with *S. obvelata* can alter host physiology, thereby confounding research results. Athymic mice infected with *Syphacia obvelata* develop lymphoproliferative disorders which can sometimes lead to lymphoma
ARTHROPODS

- **Class Arachnida (mites/ticks)**
  - Demodex musculi
  - Laelaps echidninus
  - Liponyssoides sanguineus
  - Myobia musculi
  - Myocoptes musculus
  - Notoedres
  - Ornithonyssus bacoti
  - Psorergates simplex
  - Radfordia affinis
  - Radfordia ensifera
  - Trichoecius ramboutsi
  - Trixacarus diversus
- **Class Insecta**
  - Order Diptera (flies)
    - Cuterebra sp.
    - Oestromyia leporina
  - Order Phthiraptera (lice)
    - Hoplopleura spp.
    - Polyplax serrata
    - Polyplax spinulosa
  - Order Siphonaptera (fleas)
    - Leptopsylla segnis
    - Nosopsyllus fasciatus
    - Xenopsylla cheopis
    - Cimex lectularis
  - Panstrongylus, Paratriatoma, Rhodnius, Triatoma
Myobia musculi

- The degree of pathogenicity to mice varies among mouse strains. Nude and other hairless mice are not susceptible. Strains derived from C57BL/6 or NC/Jic mice are genetically predisposed to more severe forms of disease. Gross skin changes vary from unapparent to mild dermatitis to more severe lesions that
- include ulceration and pyoderma. Lesions are most common on the head, neck, shoulders, and flank. There appears to be no direct relationship between mite numbers and severity of lesions.
Myocoptes musculinus

- Pathologic changes are often not observed in the skin of mice infested with *M. musculinus*. Hairless mice are not susceptible. Some conventional, inbred strains such as the C57Bl/6, and less commonly the BALB/c, develop pruritic dermatopathology typically associated with allergic-type hypersensitivity. Lesions include ulcerative dermatitis, erythema, lymphadenopathy.

- Twenty out of 100 mice were referred to the clinics of the Department of Internal Medicine, Faculty of Veterinary Medicine University of Mehmet Akif Ersoy, for a diagnosis. Alopecia and severe itching were observed all over the body, particularly on the abdominal area.

- Skin scrapings were obtained from affected sites of the skin for a parasitological examination. At the examination of the skin scrapings, treated with 10% of potassium hydroxide, numerous mange agents and eggs were observed. Agents were diagnosed as *Myocoptes musculinus* according to their morphological characteristics. Five mice were euthanatized and examined pathologically.

- At the histopathological examination of the skin samples taken from alopecia and pruritic areas, numerous mange agents were seen in the tunnels in the epidermis. Mild inflammatory reaction including lymphocyte and neutrophil leukocytes were observed in the dermis. After diagnosis, mice were treated with ivermectin (Vilmectin) solution via drinking water (8 mg/L drinking water once a week for 3 wks). All animals were successfully recovered after the treatment.

- Sima SAHINDURAN, Ozlem OZMEN, Mehmet HALIGUR, Bayram Ali YUKARI-Severe *Myocoptes musculinus* infestation and treatment in laboratory mice Ankara Üniv Vet Fak Derg, 57, 73-75, 2010
Myocoptes musculinus
Over the course of 2010 and 2011, mice infested with fur mites were identified in six rooms in the Whitehead Biomedical Research Building. Management of the problem requires two approaches: 1) decrease the probability of future importation of mites and manage the fur mites already on campus. Actions taken to date:

1) Mite positive rooms have been quarantined. Rooms with significant infestations were treated, with mite populations believed to have been knocked back. Degree of sterilization is unknown. Rooms with minimal evidence of infestation have been quarantined with treatment plans pending global decisions.

2) Mouse quarantine practices have been changed. Effective October, 2011, the DAR changed its quarantine practices to no longer accept live breeding pairs into quarantine. This change dramatically reduces the chance of exposing the existing census to new infectious diseases.

3) Options for rederivation have been increased. In addition to Emory’s Transgenic Mouse Facility (TMF), the DAR now offers sperm rederivation and cryopreservation services as a mechanism for mouse importation. Plans are underway to train DAR technicians in oviduct harvest to add the option of rederiving lines through fresh embryotransfer.

4) An informal survey has been sent to sister institutions to assess their experience with mouse fur mites, treatment thereof, quarantine practices, movement of mice and availability of campus services for rederivation. Responses to date are variable and reflect the complexity of the issues.
Our recommendations:

Our first recommendation has already been enacted: to change the quarantine program. We suggest staying the course here as this action dramatically reduces the chance of importing more fur mites.

Secondly, we strongly recommend the designation of the HSRB as a clean facility. This will meet investigator needs for clean mice, both on campus and for collaboration.

Thirdly and in the short term, we recommend spot-treatment with ivermectin-compounded feed and monitoring of the existing mite infestation.