ANIMAL MODELS FOR INFECTIOUS DISEASES STUDIES – PATHOGENICITY, DRUG TARGETING AND VACCINE APPROACHES It is important to understand the extent of pathogeneses and the mechanisms by which the infection is established for the development of vaccines.At the same time, Chagas was alerted by another engineer, Cornelio Cantarino Mota, to the presence in the region of a hematophagous bug known as "barbeiro" (barber bug or kissing bug) from the Triatominae family. Chagas collected and dissected some of these insects and, in the digestive tract of the bugs, found protozoan forms that he initially thought to be T. minasense in another stage of the life cycle. Chagas sent some live bugs to the Instituto Manguinhos (now, Instituto Oswaldo Cruz/RJ) and asked Oswaldo Cruz to feed marmosets kept under clean conditions with the insects. Subsequently, flagellates were detected in the controlled marmoset blood. Back in Manguinhos, Chagas then examined the flagellate samples and realized that it was not T. minasense, but another new species he called T. cruzi after his mentor Oswaldo Cruz. Suspecting that the parasite could also infect humans, Chagas went back to Lassance and started to examine the blood of patients, finally connecting the presence of T.cruzi with the symptomatology of Chagas' disease. It is remarkable to note that this classic demonstration of the etiological agent of a disease, as well as of the transmission cycle and identification of possible vectors and reservoirs, took place in early 1900's. Although enormous progress has been made in the understanding of the relationship between T. cruzi and the host, few drugs have been developed for the treatment of Chagas' disease to date and no vaccines. All treatments available for the disease are a result of studies that essentially used animal models. Leishmania is another protozoan responsible for a re-emergent disease in Brazil and in other regions of the world. Leishmaniasis consists of a complex framework of clinical signs. The World Health Organization estimates a worldwide prevalence of 12 million cases of leishmaniasis, with an annual mortality of 60,000 people and an at-risk population of approximately 350 million people in 88 countries around the world (http://www.who.int/leishmaniasis/burden/en/). Leishmania is also transmitted by an insect bite in this case the vectors are sandflies belonging to the Psychodidae family. Many mammal species can harbor the parasite. Sylvatic mammals constitute reservoirs for the disease, complicating the epidemiological control. Domestic animals, like horses, cats and mainly dogs are infected and contribute to the transmission to humans. In the mammal host, Leishmania is an obligate intra-cellular parasite found mainly in macrophages which, together with monocytes, constitute the body's phagocyte system. To survive and replicate in this defense cell, the parasite evades the humoral immune response against Leishmania produced by the host, by residing within the phagocytosomes of the macrophages. One of the strategies used to fight against invaders is the activation of oxidative burst, induced with the phagocytosis of these organisms (Cunningham 2002). Nitric oxide synthase 2 (NOS2) is also a very important enzyme in macrophage response, and when activated produces citrulline and nitric oxide (NO) from the oxidation of L-arginine: NO is a highly reactive, effector molecule which can combat invasive microorganisms (Qadoumi et al. 2002). Thus, a good animal model to be used in Leishmania studies must fulfill all these features. The chosen model uses inbred mice, specifically, the resistant C57BL/6 that produces a T helper (Th) 1 immune response and the BALB/c susceptible mice that produces a Th2 response (Sacks and Noben-Trauth 2002). The Th1 response promotes NOS2 activation and NO

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production (Bogdan 2001) and the Th2 response is associated with the production of IL-4 and IL-13 (Sacks and Noben-Trauth 2002). In respect to NO production, the amino acid arginine was shown to be a key molecule in the success or failure of the parasite in establishing the infection. In addition to being a substrate of NOS2, arginine can also be used by arginase I to produce urea and ornithine, the last being precursor of the polyamines involved in the replication of the parasite. It is interesting to note that in a Th2 response, arginase I is also activated. Leishmania itself presents arginase activity, and in a construction of a parasite with the arginase knocked out, it was possible to show that Leishmania arginase is related to parasite replication and survival (da Silva et al. 2012, da Silva and Floeter-Winter 2014). In relation to NO/ornithine physiological duality, it is interesting to note that the healing of human cutaneous leishmaniasis is associated with a Th1 response (Alexander and Bryson 2005), corroborating the observation of the mice model. However, in humans, the participation of NO in killing the parasite is still an open question, which has been addressed with the use of "humanized" mice such as the NSG strain that does not have B and T cells from mice, and also presents a higher rate of engraftment with human hematopoietic cells (Wege et al. 2012).Thus, initial efforts focused on the use of non-human primate models infected with a SIV (simian immunodeficiency virus), an HIV-like virus that infects Rhesus monkeys, producing clinical signals similar to human AIDS.To incontestably prove the correlation between ZIKV infection and microcephaly, Brazilian researchers chose the SJL mouse model, a strain defective in its ability to suppress T- cells (Hutchings et al. 1986) and notorious for its susceptibility to virus infections (Dahlberg et al. 2006).By infecting pregnant SJL females with a Brazilian ZIKV isolate, Cugola et al. (2016) demonstrated that the offspring presented clear signs of underdevelopment as well as a higher viral load in the brain when compared to mock-infected pups.These studies have investigated acute infections, lethal neonatal challenges, immune-commitment, as well as the main clinical signs of infection, such as arthritis, myositis and chronic development of peripheral joint lesion and muscle pain (Haese et al. 2016).In addition to mice models, CHIKV is also being investigated using the old world monkey group belonging to the genus Macaca, such as the Rhesus (M. mulatta), Bonet (M. radiate) and Cynomolgus (M. fascicularis) monkeys (Haese et al. 2016).However, in 2007 an outbreak of the infection occurred in Micronesia and patients presented fever, headache, anorexia, maculopapular rash, conjunctivitis, and arthralgia, but in all cases the clinical condition was, again, considered mild, self-limiting, and non-lethal (Lanciotti et al. 2008).This association produced an ongoing ZIKV epidemic in Brazil, with dramatic clinical consequences that correlated ZIKV infections and microcephaly cases (de Fatima Vasco Aragao et al. 2016).Larocca et al. (2016) achieved immunity protection against the Brazilian ZIKV in mice models using different vaccination protocols (ZIKV DNA-based vaccine and purified formalin inactivated virus vaccine).Furthermore, the use of tri-dimensional cerebral organoids derived from stem cells, simulating the first trimester of neurodevelopment in humans, also confirmed the reduction of cortical cells and the apoptotic phenotype.Infections of these tissues with the African strain of ZIKV did not show those signals, indicating that the Brazilian strain suffered a mutation/adaptation that led to the microcephaly condition (Cugola et al. 2016).In addition to the above described immunocompromised mice models, recently a study used the classical C57BL6 mice to show how an immunocompetent mammal activated

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the innate response and induced an antiviral T cell response in ZIKV infection.Another non-human primate model used in efforts to refine and develop new treatments for AIDS and HIV infection, the sooty mangabey, is naturally infected with a strain of SIV and is the source of HIV-2, a less-virulent strain of HIV.In 2013, an Asiatic strain of ZIKV made its way to Brazil and became established in a large population of Aedes aegypti, a highly competent and anthropophilic vector species.In addition to the low cost and ease of maintenance, the existence of a large panel of commercial antibodies, established lineages that provide colonies with the same genetic background, and the possibility of choosing genetically modified animals has led researchers to study the CHIKV in mouse models.EUKARYOTIC PATHOGENS The list of viruses or bacteria involved in human diseases is long: here we give a few examples on how animal models have been used to understand virus/host interactions and provide solutions, such as treatments and prevention strategies.Chagas was in a small town in the countryside of Minas Gerais (later named Lassance, after the engineer Ernesto Lassance Cunha) working on a campaign against malaria during the construction of an extension of the Central do Brazil Railroad.First, humanized mice were obtained by transplantation of human cells or tissues in partially irradiated immune-deficient animals (Shultz et al. 2012).ZIKV originated from the Zika forest - Uganda, and was initially isolated from a yellow fever sentinel Rhesus monkey (Macaca mulatta) in 1947 (Dick et al. 1952).Artificially feeding Aedes aegypti mosquitoes on infected mice and monkeys confirmed that the transmission is made by the bite of mosquitoes from the Aedes genus (Boorman and Porterfield 1956).This emphasizes the fact that animal models related to viral replication are still warranted in order to promote the understanding of the immunological responses that can lead to the development of drugs and vaccines (Ozden et al. 2007).This practice is so efficient that the World Health Organization has produced a complete vaccination schedule for infants and adults against many biological agents: (http://www.who.int/immunization/policy/immunization_tables/en/).Another study also using the immunocompetent C57BL6 showed windows, that could be correlated to human embryogenesis when infection caused the congenital abnormalities that result the microcephaly picture (Xavier-Neto et al. 2017).ZIKA VIRUS The Zika virus (ZIKV) is an RNA containing virus belonging to the Flaviviridae family, related to yellow fever, dengue and West Nile viruses.Further investigation of ZIKV-infected mice brains showed cortex malformation with reduced cell number and cortical layer thickness.Another alternative approach was the use of two-dimensional neural cell culture, neurospheres and cerebral organoids, that, when infected with ZIKV revealed cell death and a significantly smaller size compared to control cells.The efforts in achieving an effective vaccination protocol has led the Butantan Institute in Sao Paulo (BR) to concentrate their efforts on the production of the DNA vaccine, with a promise of getting to a first round of human tests in 2017.The higher level of complexity in these organisms besides the many mechanisms they employ for evading host responses have made it impossible to develop effective vaccines for eukaryotic organisms.In the early 1900's, Carlos Chagas, a bright young Brazilian physician and researcher, described the etiological agent as well as the mode of transmission of Chagas' disease using an animal model.This dual-model (virus/host) has been used to develop a better understanding of the pathogenesis of the virus in order to develop drugs and preventive strategies, such as the development of vaccines (Micci and Paiardini 2016).Beyond the understanding of the pathophysiology of

ZIKV infection, animal models are also being used for the development of therapeutic targets and vaccines for the control and prevention of ZIKV infections.The same research group also observed promising results with the two immunization protocols in Rhesus monkeys (Abbink et al. 2016).It is historically known that the first human vaccine, developed by the young British physician Edward Jenner, was a result of his observation of dairymaids who used to handle cows in the milking process.The work of Louis Pasteur in developing the rabies vaccine, for both dogs and humans, is another example of the efficiency of vaccination in protecting against virus-caused diseases.Antibiotic therapy has helped to control many of them, and, just like for viruses, protection and prevention from some bacteria-caused diseases can be obtained through vaccination.The virus is not able to infect mice, rats, rabbits, or macaques, although it can replicate in chimpanzees (Victor Garcia 2016).Thanks to non-human primate research on AIDS and HIV, a leading HIV/AIDS preventive vaccine is now licensed and in phase 2a human clinical trials.Although the ZIKV was widely spread in Africa and Asia, the clinical aspects of the infection were considered mild, with a low incidence of human infection.Cugola et al. (2016) also used alternative cell cultures to correlate ZIKV-infection and microcephaly phenotype.ZIKV-infected human pluripotent stem cells derived from neural progenitor cells showed apoptotic cell death.The most prominent problem is the recurrent appearance of arthralgia that might compromise the quality of life of the affected individual (Couturier et al. 2012, Schilte et al. 2013).Thus, Chagas took a careful look at that organism and described a new species, named Trypanosoma minasense.This inspired Jenner to inoculate the cowpox virus as a protective practice against smallpox (Riedel 2005).The Jenner vaccine was very important to mankind, and resulted in the eradication of smallpox.In the following section, we will give some examples on how animal models shed light onto the etiology of infections, leading to treatment, prevention and control protocols.In the end of the last century, one of the most remarkable and dramatic events in terms of public health was the advent of the acquired immunodeficiency syndrome (AIDS) epidemic that killed millions of people, especially in at-risk populations.With the lack of an efficient vaccine, the search for chemotherapy treatment for patients infected with human immunodeficiency virus (HIV) became urgent.In addition to primate research, other animal models have been used to address the most relevant aspects of HIV infection, mostly virus persistence.From this initial effort, different humanized mice models have been developed and are now being used to advance the HIV/AIDS field (Victor Garcia 2016).It was only isolated from a human in 1968 (Moore et al. 1975, Fagbami 1977), who clinically only presented fever.The macaque model is especially relevant to the study of CHIKV pathogenesis and the efficacy of drugs and vaccines.This highlights the relevance of animal models that can lead to a better understanding of the host/pathogen relationship, and ultimately ways to control these infectious agents.Examining a blood sample of a marmoset (Callithrix penicillata), he noticed the presence of a protozoan of the Trypanosomatidae family.Jenner observed that because these women had had cowpox, they were never infected with smallpox.HIV/AIDS Although vaccination is the best choice for controlling viruses, the development of a vaccine is not always easy or possible.Antiretroviral treatments are now very effective in suppressing HIV replication.Those observations are consistent with human microcephaly (Cugola et al. 2016).The study also showed a new epitope in the ZIKV envelope that is recognized by CD8+ T cell, an important finding for vaccines

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development (Pardy et al. 2017).The authors also showed that the protection could be achieved against the Puerto Rico ZIKV isolate as well.It is a Togaviridae RNA virus also transmitted by Aedes bites.The symptoms of acute infection in humans (i.e. fever, headache, myalgia, fatigue and polyarthalgia), although painful, resolve in a few days.In addition to viruses, bacteria can also be the etiological agent of many human or animal diseases.The major problem with HIV is its elevated human tissue tropism, making it difficult to model the condition in animals.CHIKUNGUNYA VIRUS Another important virus that is currently a subject of public health concern in Brazil is the Chikungunya virus (CHIKV).Mice present many advantages as models for CHIKV infection.The study and development of preventive tools for eukaryotic organisms is particularly problematic.At that time, similar organisms had been implicated in the cattle disease Nagana as well as in human sleeping sickness in Africa.The chronic symptoms are due to the ability of the virus to replicate in joint tissues and muscle cells.Despite the global spread of the virus, affecting millions of patients, its pathogenesis is still unknown.Chagas had an interest in research, and used to collect and examine the local fauna.However, treatment interruption leads to a resurgence of AIDS.Therefore, the first vaccine was only possible due to the use of a living model.As already mentioned in this review, it is important that the appropriate model is used to provide the required conditions to answer a specific scientific question.

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