Relationship of parasites and pathogens diversity to rodents in Thailand
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ABSTRACT

Rodents have proven to be of increasing importance in transmitting diseases to humans in recent decades, through the emergence of worldwide epidemics and, in Thailand, through the emergence of leptospirosis and scrub typhus. Investigations of parasites and pathogens in murine rodents have helped to describe the implication of the main species and understand the different ways of transmission. From wild to anthropized habitats, rodents can be reservoirs, hosts or vectors of infectious organisms. Related species can react very differently to the same pathogens, with pivotal implications for the understanding of their natural circulation. Scrub typhus is transmitted to humans through the bites of trombiculid mites that have previously fed on infected rodents, generally occurring in wild habitats. Leptospirosis can affect people without any direct contact with infected rodents, but by indirect spread in agricultural areas. Parasitic diseases, such as toxoplasmosis and trypanosomiasis benefit from the proximity of rodents to domesticated animals to jump from one vector to another before reaching humans. By occupying almost all biotopes and by rapidly adapting to environmental changes, rodents are fundamental in the maintenance and transmission of an impressive number of infectious organisms to humans.

Key words: rodents, zoonoses, parasites, toxoplasmosis, trypanosomiasis

INTRODUCTION

About 70% of emerging infectious diseases imply a vector in their transmission cycle (Gratz, 2006). These vectors can be insects (mosquitoes, cockroaches, sandflies, fleas, lice, triatomines, midges, etc.), acarines (ticks and mites), but also mammals. Within mammals,
rodents, which represent 40% of mammalian species (Wilson and Reeder, 2005), have been pointed out as the major host and vector of zoonoses since they have been responsible for several regional and worldwide epidemics that have gone down in history. One of the oldest identifiable diseases known to man - the plague, uses fleas as vectors and rodents as natural hosts. The Plague of Justinian afflicted the Byzantine Empire in the years 541–542 AD and contributed to its waning (Little et al., 2006).

The Black Plague, also known as the Black Death, later affected the region from central Asia to Europe, starting in the 14th century and has been one of the deadliest pandemics in history. More recently hantaviruses spread by rodents were first described during the Korean war in the 1950s, and have later caused several epidemics in Asia, Europe and America (Schmaljohn and Hjelle, 1997). While the progress in medicine and epidemiology has helped to identify a growing number of diseases associated with rodents, still little is known about their real incidence worldwide, as Gratz already mentioned in 1974. Rodent-borne diseases are numerous; they can be bacterial (e.g. leptospirosis, plague and rickettsioses: scrub typhus, murine typhus), parasitic (e.g. leishmaniosis, babesiosis, schistosomiasis) or viral (hantaviral infections, arenaviral infections). Rodents can play different roles in maintaining or spreading the diseases: they can be major or accidental reservoirs, hosts or vectors, depending on the disease and location. Thus, rodents can be part of the transmission of diseases to humans without direct contact with them and even more being far away from the place of infection.

A key reason for the importance of rodents in the transmission of zoonoses relies on their ecology. Rodents are present in most of biotopes on all continents other than Antarctica, being able to breed rapidly, eat a large variety of food and then adapt to fast environmental changes (Carleton, 1984). Their populations move following the food and shelter availability and, therefore, their local dynamics answer to human practices, especially agriculture. Rodents can conquer new territories such as deforested areas or even new human settlements in remote areas. In countries like Thailand, where the hunting pressure is high, rodents constitute most of the wildlife. Thus, in proximity to humans are living rodents, which constitute a potential threat for human health through the pathogens they carry.

The way of transmission can be multiple: from any biotopes inhabited by rodents, from the wildest to the most anthropized ones, rodent-borne parasites and pathogens can find their way to reach humans. This article proposes to illustrate the different roles of rodents in this transmission, through examples of major health concern in Thailand.

From wild biotopes to humans - the role of rodents in the transmission of scrub typhus

Although wild rodents may have no contact with humans, their presence may help to perpetuate parasites and pathogens that pose a health threat to people. These rodents can be a wild reservoir or vector horizontally transmitting infectious organisms to other rodent species living closer to anthropized environments. They can also be a wild reservoir, in which intermediate vectors, usually insects or acarines, feed and get infected before transmitting these infectious organisms to humans. In the case of scrub typhus transmission, rodents are considered the main host of pathogenic bacteria and can occur in biotopes far from human presence, while acarines make the link by feeding on rodents and biting people.

Scrub typhus was first described by the Chinese about 2000 years ago, and is nowadays endemic in the Asia-Pacific region (Kawamura et al., 1995). It is transmitted to humans by infected chiggers, the larval stage of trombiculid mites (Leptotrombidium spp.) feeding on wild and
domestic rodents. Humans are accidental hosts in this zoonotic disease. This illness is caused by *Orientia* (formerly *Rickettsia* *) tsutsugamushi* (Tamura *et al.*, 1995, Dumler *et al.*, 2001), an obligate intracellular gram-negative bacterium, which was first isolated in 1930 (Kawamura *et al.*, 1995). Even though it has been recognized as one of the tropical rickettsioses diseases, *O. tsutsugamushi* has a different cell wall structure and genetic composition more than that of the rickettsiae (Tamura *et al.*, 1995, Dumler *et al.*, 2001).

The larva is the only stage that can transmit the disease to humans and other vertebrates. These tiny chiggers attach themselves to the skin. During the process of obtaining a meal, they may either get infected by the *O. tsutsugamushi* pathogens from the host or transmit them to other mammals or humans. The term “scrub” was used to describe the type of vegetation characteristic of the first observed human epidemics. Infections have later been reported from different biotopes, often associated with disturbed ecosystems.

Scrub typhus is estimated to cause about one million cases annually worldwide. However, the similarity of symptoms with other tropical fevers, especially dengue and leptospirosis, may result in the misdiagnosis of these diseases and also hide possible co-infections (Watt *et al.*, 2003a and b). In Thailand, scrub typhus has been an endemic disease with a low incidence till the 1980s. In 1985, the incidence started to increase to 5,094 cases in 2001 and has been reported to be decreasing since then (Thai Ministry of Public Health).

Several murine rodents have serologically been tested positive for *O. tsutsugamushi*: *Bandicota indica*, *B. savilei*, *Berylmys berdmorei*, *Niviventer sp.*, *Rattus andamanensis*, *R. argentiventer*, *R. exulans*, *R. losea*, *R. norvegicus* and *R. tanezumi*, the asian lineage from the *Rattus rattus* complex (Strickman *et al.*, 1994; Imvithaya *et al.*, 2001; Coleman *et al.*, 2003). Other species, and especially wild rodents, may not have been tested in sufficient number to conclude that they can or cannot be, a vector of scrub typhus. Two distinct research studies revealed very high seroprevalences for *Rattus tanezumi* with 29.2% (123/421) positive in different provinces across the country (Imvithaya *et al.*, 2001), and 22.5% (419/1,863), also in different regions (Coleman *et al.*, 2003). Other species showed lower but still important incidences: *R. losea* with 12.9% (82/638), *B. indica* with 13.4% (101/755), *R. exulans* with 4.3% (20/465) and *B. savilei* with 3.3% (1/30). A very high prevalence was surprisingly reported from *R. norvegicus* 32.4% (11/34), which occur in urban areas, out of the usually-described places of infection (Imvithaya *et al.*, 2001).

Even though scrub typhus epidemics have been described in wild environments, serological investigations have proved that it remains widespread among rodent populations. As these rodents inhabit different habitats in wild or domestic environments, scrub typhus represents a real threat to human health.

**From agricultural areas to humans, the role of rodents in the transmission of leptospirosis**

In agricultural areas, people and rodents share the same space, the farmers working during the day and the rodents looking for food from dusk to dawn. By spreading infectious organisms along their tracks, rodents can indirectly transmit diseases to people, as happens for leptospirosis.

Considered to be the most common zoonosis in the world, leptospirosis mostly occurs in tropical and subtropical countries, where high rainfall helps the transmission of the pathogenic bacteria. Leptospirosis is caused by spirochaetes of the genus *Leptospira*, directly or indirectly transmitted from animals to humans (Faine, 1999; WHO, 2003). It has emerged in Thailand since 1997, as a major health concern (Bharti *et al.*, 2003). From agricultural areas to humans, the role of rodents in the transmission of leptospirosis
After eight years of epidemics, affecting thousands of people yearly with the highest incidence in 2000 (14,285 cases according to the Thai Ministry of Public Health), the incidence of leptospirosis has decreased and stabilized to the satisfaction of public health officers and local communities (Herbreteau, 2007). With a total of 1,202 deaths from 1997 to 2004, mainly in the Northeast, North and South, leptospirosis has been raised as a major threat in agricultural villages.

Leptospirosis is considered as an occupational disease, affecting mostly farmers working in flooded rice fields, where they get infected through skin lesions. Identifications of *Leptospira* spp. have shown a huge number of different serotypes in both rodents and humans (Boonyod *et al.*, 2001; Kositanont *et al.*, 2003), and, considering that, after infection, serovar-specific antibodies do not protect against infections with other serovars (WHO, 2003), immunity is a limiting factor in the resistance to the disease. *Leptospira* spp. have proved to have a remarkable survival ability of up to several weeks or months in a wet environment and alkaline soils (Henry and Johnson, 1978; Smith and Self, 1955; Smith and Turner, 1961; Faine *et al.*, 1999), making the environmental conditions the major limiting factor to the transmission to humans. Leptospirosis is then a seasonal disease, amplified in incidence during and after the rainy season, from June to October and later in the southern region.

A wide range of animals, small or large mammals, birds, reptiles or even ticks are potential hosts and vectors. In Thailand, main vectors are murine rodents, spreading the bacteria through their urine or feces in the environment (Plank and Dean, 2000). A high prevalence was found in *Bandicota indica*, a large rat occurring in rice fields: 13.6% (36 positive for 265 tested) revealed positive in Northeastern Thailand (Phulsuksombati *et al.*, 2001; Doungchawee *et al.*, 2005). *Rattus tanezumi* also demonstrated high seroprevalence with 7.4% (50/676) in Northern and Northeastern regions (Bunnag *et al.*, 1983) and 5.0% (23/464) in different provinces across the country (Imvithaya *et al.*, 2001). Furthermore, as for scrub typhus, a very high prevalence, 41.4% (127/307), was notified from the cosmopolitan rat, *Rattus norvegicus*, present in Bangkok metropolitan area, where only a few human cases have been reported (Phulsuksombati *et al.*, 2001; Doungchawee *et al.*, 2005). The main vectors of leptospirosis may not be the rodents having the highest seroprevalence, but those living in closer proximity to humans and occurring in the places of infection, mainly ricefields. *Bandicota indica*, which is the majority murine rat occurring in Thailand, can be considered as a principal vector, all the more so as it is hunted for its meat. Both *Rattus tanezumi* and *R. exulans* may also be major vectors as they can live around and inside houses.

**Within anthropized environments, the role of rodents in the transmission of parasitic diseases i.e. toxoplasmosis and trypanosomiasis**

Living in anthropized environments, rodents share their territories with domestic animals and contribute to the transmission of parasites and pathogens between animal species and humans.

**Major impacts of parasitic diseases**

Domesticated animals, pets (i.e. dogs, cats) and livestock (i.e. cows, pigs, etc.), have been associated with human society for thousands of years and it is difficult to think of human enterprise throughout history without the contribution of animals. Domestic animals serve as companions to humans, a mean of transportation or work in many countries and a source of food for a world with a rapidly-growing human population. In addition, wild animals are just as important in maintaining a balance in nature and the preservation of ecosystems. Parasitic-borne diseases are extremely harmful to animals and
cause severe losses. Losses are not only the result of animal mortality, but also due to morbidity causing a decrease in productivity of meat production, milk yield or egg laying and the cost of control measures.

The consequences of parasitic-borne diseases are numerous and diverse. They range from economic loss related to the production of farm animals to disability and the loss of work years and most severely to the loss of human lives. Therefore, the drivers influencing parasitic-borne diseases can have devastating effects upon the lives of people of every nationality or socioeconomic class. Parasitic diseases are responsible for more than 25% of the global human disease toll. Of the ten priority infections on the WHO list, eight are parasitic-borne diseases (Harrus and Baneth, 2005). These diseases are responsible for a large number of disability-adjusted life years, a measure of the number of healthy years of life lost due to premature death and disability (WHO, 2002).

**Toxoplasmosis**

*Toxoplasma gondii* is an intracellular apicomplexan protozoan with a world-wide distribution, capable of infecting a variety of animals. *T. gondii* can also infect humans, and warm-blooded domestic and wild animals such as birds and rodents. Warm-blooded animals, including humans and rodents, are intermediate hosts that harbor tissue cysts in their bodies. Rodents can present a high prevalence via oocyst contamination, and also act as persistent, intermediate host reservoirs for *T. gondii* through vertical transmission. Infection by *T. gondii* is estimated to infect 30% of the human population (Aspinall *et al.*, 2002). Although the infection usually does not cause a significant problem for healthy individuals, it can be life-threatening for congenitally-infected and pharmacologically-immunosuppressed patients (Chintana *et al.*, 1998). Serological studies in Thailand have shown widespread cases of infection in humans (Pradatsundarasa and Papasarathorn, 1966; Maruyama *et al.*, 2000), dogs and cats (Jittapalapong *et al.*, 2006), swine (Sriwaranard *et al.*, 1981; Nishikawa *et al.*, 1989; Tuntasuvan *et al.*, 1989), goats (Jittapalapong *et al.*, 2004), elephants (Tuntasuvan *et al.*, 2001), tigers (Thiangtum *et al.*, 2006) and rodents (Jittapalapong *et al.*, 2006).

Cats are important in the natural life cycle of *T. gondii* because they are the only hosts that can directly spread *T. gondii* in the environment. Thus, cats can recycle and amplify the infection by releasing millions of infected oocysts into the environment. Furthermore rats are considered the main reservoir for this protozoan parasite since they can live in proximity to humans. Recent studies have demonstrated that wild rodents can represent not only a highly prevalent, but also a persistent intermediate host reservoir for *T. gondii* (Jackson *et al.*, 1986; Webster, 1994). However prevalences in rodents may present regional variations. In urban areas in Panama city, *Rattus norvegicus* demonstrated a high seroprevalence (23.3%) with 52 positives out of 226 tested (Frenkel *et al.* 1995), while Dubey and Frenkel (1998) summarized the worldwide prevalence of *T. gondii* in different species of rats and concluded that the prevalence of viable *T. gondii* in *Rattus norvegicus* was relative low.

Recent investigations in Thailand showed an overall 4.6% seroprevalence of *T. gondii* infections in 461 rodents trapped in field crops, forests and urban areas over 11 provinces (Jittapalapong *et al.*, 2006). Rodents were classified as *Sciuridae* (*Menetes* and *Callosciurus*) and *Muridae* (*Bandicota*, *Berylmys*, *Leopoldamys*, *Maxomys*, *Mus*, *Niviventer* and *Rattus*). *T. gondii* infection was highly prevalent in a wild rodent, *Leopoldamys sabanus* (12.5%), compared to other species. The relatively-high incidence of *T. gondii* infections in rodents underlines the risk for cats to become infected by predation on infected rodents (Ruiz and Frenkel, 1980). Rodents might be an
important linkage for disease transmission via the food chain. This result will be beneficial for control strategies of toxoplasmosis and other rodent-borne diseases. This fact must be explained by natural oscillations in the parasite-host systems. This was observed with cockroaches, which may serve as mechanical vectors of \textit{T. gondii}, through ingestion of oocyst-containing cat feces. Rodents also become infected when they consume contaminated cockroaches (Smith and Frenkel, 1978).

The diversity of carrier-rodent species demonstrates the presence of toxoplasmosis in different biotopes and the wide transmission between species. The global environmental changes observed in Thailand may have modified the biodiversity of these parasitic pathogens.

**Trypanosomiasis**

The etiologic agents of many serious, infectious diseases utilize invertebrate hosts during a portion of their life cycle. Most of these agents are adapted to hematophagous arthropods that share their vertebrate hosts. The identification of these arthropod vectors and vertebrate reservoirs is usually a key to sustain an efficient control of vector-borne diseases. Trypanosomes are flagellate protozoan parasites, some of which can cause distinct zoonoses, the most notable of which include the Leishmaniases, American trypanosomiasis (Chagas’disease) and African trypanosomiasis (sleeping sickness). Like most vector-borne zoonotic agents, trypanosomes that infect humans utilize a broad, vertebrate host range and a relatively-narrow range of invertebrate vectors. However, there are rare yet notable exceptions to the latter generalization.

The genus \textit{Trypanosoma} can be divided into two major groups that infect vertebrates - the salivaria and the stercoraria (Hoare, 1964). Members of the salivaria or ‘anterior station’ group are frequently pathogenic to vertebrate hosts. These organisms usually undergo cyclical development in the anterior insect midgut prior to biological transmission via vector salivary glands. Some members of this group are mechanically transmitted by inoculation during vector feeding and some others are completely adapted to vertebrates without the need for an invertebrate vector. Conversely, most members of the stercoraria, or ‘posterior station’ group, are nonpathogenic to natural vertebrate hosts. These parasites undergo cyclical development in the arthropod hindgut before transmission to the vertebrate hosts through vector feces. Both \textit{Trypanosoma} groups are enzootic to Thailand.

Trypanosomes enzootic to Thailand include \textit{T. evansi}, a salivaria, which is considered the primary agent of trypanosomiasis among domestic animals in Asia and India. \textit{T. evansi} is mechanically transmitted among bovids, camelids, cervids, equids and canids by biting flies in the suborder \textit{Brachycera} (Shrivastava and Shrivastava, 1974; Joshi et al., 2005). Notably, \textit{T. evansi} was recently reported to cause a case of human trypanosomiasis in India (Joshi et al., 2005). Stercoraria in Thailand include \textit{T. lewisi-like} species of the subgenus \textit{Herpetosoma}, which are generally vertebrate-specific, non-pathogenic flea-borne parasites of rodents. Although \textit{Herpetosoma} species are considered specific to a single vertebrate host genus, they reportedly infect a relatively-broad range of flea vectors (Molyneuz, 1969; Linardi and Botelho, 2002; Desquesnes et al., 2002).

Human trypanosomiasis associated with \textit{Trypanosoma} spp. enzootic to Thailand is considered extremely rare, thus the recent finding of a \textit{T. lewisi-like} (\textit{Herpetosoma}) infection in an infant compelled an investigation to survey different rodent populations for similar infections (Sarathapan et al., 2007). Recent surveys on rodents could identify \textit{Herpetosoma} in \textit{Rattus} (14.3 %) and \textit{Bandicota} (18.0 %) rodent species and salivarian trypanosomes in \textit{Leopoldamys} (20 %) and \textit{Rattus} (2.0%) species (Jittapalapong et al., 2007). \textit{Herpetosoma} were prevalent among...
rodents associated with both human and sylvatic habitats, while three of four salivaria-positive rodents were from a forest biotope. A Herpetosoma ITS1 sequence amplified from one of these samples was 97.9% identical to that reported for T. lewisi in an experimentally-infected rat and 96.4% identical to the sequence amplified from blood from a Thai infant.

Fleas are often opportunistic ectoparasites of available mammalian hosts. Thus, a rodent reservoir and flea vector appeared to be the most likely source of a T. lewisi-like (Herpetosoma) infection recently reported in a sick infant from Thailand (Sarataphan et al., 2007). R. exulans, closely associated with humans, was surprisingly identified as one of the Herpetosoma reservoirs. Although fleas that feed on R. exulans are more likely to come into contact with human hosts, the rarity of presumably flea-borne human trypanosomiasis indicates that the field rat hosts identified in this study, B. indica and B. savilei, also warrant consideration as possible sources of human infection with the T. lewisi-like parasite detected in the Thai infant.

Trypanosomes, presumably T. evansi, have been routinely detected in buffaloes, cats, dairy cows, dogs, elephants, pigs, horses and rodents all over Thailand (Booyawong et al., 1975; Chauchanapunpol et al., 1985, 1987; Loehr et al., 1985, 1986; Mathias and Muangyai, 1980; Nilkhamhang, 1980, 1985; Nishigawa et al., 1990; Patchimasiri et al., 1983; Rodthian et al., 2004; Sarataphan et al., 1986; Jittapalapong et al., 2007), and T. lewisi-like parasites were reported in rats from Chiang Mai province (Natheewattana et al. 1973). T. lewisi and other Herpetosoma are commonly found in the blood of rats worldwide, and members of this subgenus are generally considered to be non-pathogenic and rarely found in humans (Hoare 1972). The results of this survey on Thai murine rodents confirmed that some wild species could represent highly prevalent reservoirs of T. lewisi-like parasites and that habitats significantly affect the prevalence of T. lewisi. It suggests that the degree of anthropization may influence the transmission of Trypanosoma spp.

Trypanosoma subgenera generally utilize different vertebrate and invertebrate hosts. In Thailand, different rodent species have partially overlapping distributions in various habitats, thus the natural vertebrate reservoir(s) of the recent human infection cannot be identified using current information. Although rodents found in human habitats may be suspected, given the rarity of human trypanosomiasis in Thailand, it is also plausible that the aforementioned human infection was a Trypanosoma sp. naturally infecting rodent species not normally associated with humans. This was accomplished with the same PCR assay used to characterize the infant infection (Sarataphan et al., 2007). Within twelve examined rodent species, three included positive individuals for stercorarian trypanosomes. Also, four rodent individuals were found infected by salivaria trypanosomes. Detection of salivaria trypanosomes in these rodents was unexpected, and, although only a few rodents were positive, it is noteworthy that all but one were collected from a forest habitat and that two out of the five L. edwardsi collected were positive for this group.

**DISCUSSION**

Reviewing the major studies of rodents, pathogens and parasites reveals first the difficulties in identifying rodents to the species level. A correct identification is nevertheless a prerequisite to the interpretation of the rodent ecology and further role in the transmission of infectious organisms. The high diversity of rodents in Southeast Asia and the lack of taxonomic studies have made this identification hazardous. Medical researches dealing with rodent-borne diseases are generally using an out-of-date taxonomy leading to misinterpretation of the disease’s ecology. Also these studies have been restricted in Thailand to
the most common species with special emphasis on domestic species. This choice has been directed by an intuitive assumption that the rodents responsible for the transmission of diseases were those living closely to humans and also by the ease in catching those species. Therefore little is known about the wild species, even though they may have a key role in maintaining pathogens and parasites through the seasons and local environmental changes.

Furthermore, investigations have been limited in Thailand to the most common infectious agents, with a known impact on human health, as for leptospirosis and scrub typhus, or a potential threat for people, as for hantaviruses. Only recently have rodents been tested for parasitic diseases, toxoplasmosis and trypanosomiasis. Finally, little is known on other rodent-borne parasites and pathogens that can be transmitted to humans (arenaviruses, Hepatitis E virus, rabies, bartonellosis, melioidosis, schistosomiasis, babesiosis, plague, etc.). Further investigations are needed with emphasis on the parasite load to also understand the susceptibility of each species.

In a global approach, environmental changes have an effect on the biodiversity and dynamics of rodents and associated pathogens, and act on the emergence and re-emergence of parasitic diseases. These changes have been mostly driven by human pressure on ecosystems through massive deforestation and agricultural development. Deforestation and transformation of forests to grazing land, agricultural areas and human settlement result in significant alterations to the environment and changes in the composition of vectors, and therefore, the introduction of emerging or re-emerging pathogens. Changes in climate and temperatures affect the distribution of vectors, reservoirs and the effectiveness of pathogen transmission by vectors. Further integrated research will be needed to assess the local or global change effects on the populations of rodents and associated parasites.

**CONCLUSIONS**

With an increase in the incidence of rodent-borne diseases worldwide, awareness has risen of the importance to study rodent ecology and population dynamics to monitor the risk of transmission (Mills and Childs, 1998). Recent advances in genetics may soon help to resolve the taxonomy of Southeast Asian murine rodents. Further ecological studies of each population will be needed to describe their dynamics and estimate their geographical distribution. The knowledge of the distribution of the rodent vectors associated with serological surveys will help to predict the distribution of the pathogens and assess the potential risk for human health. This risk may indicate the probability of infection in a given habitat. In addition, it is important to assess the overlapping of different species distribution to understand the possible horizontal transmission of pathogens between species. Different species with overlapping distributions may get infected from each other as they favor and share the same tracks in their environment (e.g. along the edge of the fields or along the wall in houses). An important mean of transmission is through the urine that rodents leave while they move to mark their territory and that they smell to recognize the presence of other individuals. Typically, a wild species, occurring in a primary forest without contacts with humans, can be the host of a pathogen that could be progressively transmitted to other rodent species living at the frontier of the wild habitat and in closer proximity to humans. Furthermore, the study of population densities, together with their seroprevalence, would allow, in theory, an assessment of the possible onset of human epidemics. Recovering this understanding of the transmission of pathogens from rodents to humans involves several studies, including ecology, taxonomy, epidemiology and spatial analysis, whose reliability is the precondition for the quality of the final conclusions.
LITERATURE CITED


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