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Invasive Alien Squirrels (*Callosciurus erythraeus*): Impacts of the Animal Trade and Health Assessment in Northern Italy

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ABSTRACT

Biological invasions, often linked to the exotic pet trade, represent a major threat to global biodiversity and are increasingly recognized as drivers of pathogen pollution. The Pallas's squirrel (*Callosciurus erythraeus*) is an invasive alien species that has established populations in Northern Italy. Its presence raises ecological concerns, particularly because it can compete with and potentially displace the native Eurasian red squirrel (*Sciurus vulgaris*), whose presence is already threatened by the American grey squirrel (*Sciurus carolinensis*). At the same time, the introduction of a new species into an ecosystem may alter local disease dynamics by introducing new pathogens or interacting with those already present. To better understand the health status and potential epidemiological role of this invasive population, this study carried out a comprehensive pathogen screening. Necropsies were performed on 99 Pallas's squirrel carcasses collected in the province of Varese between January 10th and February 27th, 2025, as part of the regional eradication program. The results show that the Italian population of Pallas's squirrels carries a relatively low pathogen load. This finding supports the enemy release hypothesis, which suggests that invasive species may become successful partly because they leave many of their natural parasites and pathogens behind when introduced to a new environment. These results are also consistent with previous studies on this species, which suggested no evidence of pathogen spillover or spillback from invasive Pallas's squirrels to the native red squirrel species and indicated that most of the detected pathogens were likely acquired locally rather than introduced with the invasive squirrel, with the exception of the nematode *Strongyloides callosciureus*. Overall, the low diversity and prevalence of pathogens observed suggest that the current sanitary risk posed by this population is limited. However, because invasive hosts can accumulate pathogens over time and indirectly facilitate local disease transmission, continuous monitoring, expanded research - particularly concerning microparasites - and the strict enforcement of trade bans remain crucial for long-term ecological and public health protection.

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INTRODUCTION

Biological invasions, defined as the human-mediated movement of species outside their natural range, represent one of the primary drivers of global biodiversity loss and can profoundly modify native ecosystems [1]. When species are moved, intentionally or not, beyond their natural range, the introduced population can become invasive and cause significant ecological harm. Their impacts may include local species declines or extinctions, degradation of ecosystem functions, and risks to human health.

To be defined as invasive a species has to be able to establish population outside its distribution range, must have a fast demographic increase and should have an impact on native species and/or ecosystems [2].

Over the past centuries, biological invasions have increased dramatically. According to the 2023 report of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES), approximately 37,000 alien species have been introduced worldwide, with Europe representing the most invaded region and hosting more than 18,000 established alien species [3] ,[18]. Of the alien species recorded globally, more than 3,500 are classified as invasive and have documented ecological or economic impacts, although this number is likely underestimated and expected to increase [3].

The global economic burden associated with Invasive Alien Species (IAS) is extremely high, exceeding USD 423 billion annually as of 2019, with evidence suggesting a four-fold increase in costs every decade since 1970 [3], [4]. This value, however, represents a major underestimation of the real situation, since data on the economic impact of many invasive alien species are still missing [5]. The trend highlights how quickly the problem is growing and how significant the economic consequences of biological invasions have become. Recently Italy, like many other countries, has faced an increasing number of biological invasions: the average number of introduced species per year has increased exponentially over time, rising from about 6 species per year in the 1970s to 25 species per year in the current decade [6] with mammals representing a significant proportion of these introductions. Among these, squirrels constitute one of the most challenging groups since it has been proven that, once a squirrel is introduced, it has a very high potential to establish a population, both in regions close to their native range and in entirely new areas far beyond it [1]. 248 introduction events involving squirrels have been recorded worldwide, with 18 of the 20 species introduced successfully establishing self-sustaining populations [7].

One of the main threats in Europe is the potential competition with the native red squirrel (*Sciurus vulgaris*), particularly through indirect competition for resources in areas where the native and alien species coexist. Additional concerns are related to the impact on avian communities, as squirrels may prey on eggs and nestlings, [8] and huge damage is also caused by introduced squirrels to natural vegetation and tree plantations through their bark-stripping behaviour [1].

Among the previously cited risks, invasive squirrel - and IAS in general - can function as hosts or vectors of pathogens of human and animal health concern. They may reshape local infection dynamics by introducing pathogens absent from the release area with potential spillover to native species, by acquiring endemic pathogens and causing spillback to native populations, and by altering local population dynamics in ways that indirectly affect pathogen transmission [9], [10].

The grey squirrel (*Sciurus carolinensis*) represents the clearest example of an invasive species causing native declines. Introduced to Australia, South Africa, Great Britain, Ireland, Scotland and Italy, it has been closely associated with the replacement of the native red squirrel. In the UK, Scotland and Ireland, the replacement process is exacerbated by the squirrelpox virus (SQPV), a virus belonging to the parapoxvirus clade. Grey squirrels can carry the virus without developing symptoms, acting as a reservoir, whereas red squirrels typically die shortly after infection [11]. Nevertheless, the grey squirrel is only one of several introduced squirrel species. Another invasive species of European and national concern is *Callosciurus erythraeus*, the Pallas's squirrel: a medium-sized, diurnal, arboreal rodent whose natural distribution extends across much of northeastern South Asia, central and southern China, and mainland Southeast Asia, as seen in Figure 1 [8].

Figure 1. *Callosciurus erythraeus*'s native range – [Schockert, 2012] [12]

Its remarkable adaptability, including the ability to colonize urban environments, has favored its spread beyond the native range, with non-native populations now present in France, Belgium, the Netherlands, Argentina, Japan, Hong Kong and Italy (Figure 2) [12], where first confirmed sighting in Lombardy (Varese Province) dates back to 2007 [13].

Figure 2. Original range of *Callosciurus erythraeus* and areas of introduction (•) – [Schockert, 2012] [12]

As with other invasive species, the exotic pet trade represented the main pathway of introduction. In fact, both grey squirrels (S. carolinensis) and Pallas's squirrels (*C. erythraeus*) were legally sold as pets in Italy until February 2013 [14]. In response to the biological invasion a complete trade ban on three squirrel species (S. carolinensis, S. niger, *C. erythraeus*) was enacted through the Inter-Ministerial Decree No. 28 of 2 February 2013, in compliance with EU Wildlife Trade Regulation No. 338/97 (Annex B) [15]. *Callosciurus erythraeus* is also included in the Union list of invasive alien species of concern (updated 2 August 2022) under EU Regulation No. 1143/2014. This listing places the species under strict regulatory measures, including bans on keeping, importing, selling, breeding, or releasing individuals into the wild. In addition, the Regulation requires Member States to take action by addressing pathways of unintentional introduction, implementing preventive measures, ensuring early detection and rapid eradication of new incursions, and managing populations that are already widely established [16].

Currently, the presence of Pallas's squirrel in Italy is confined to Lombardy, in the mountainous area north of Varese Province, as shown in Figure 3, where it co-occurs with the native red squirrel. Ecological barriers - lake Maggiore and the Valtravaglia and Valcuvia valleys - currently limit its spread. However, overcoming these barriers could enable the species to expand beyond Italy's borders, [8] extending invasion risks to an increasing number of areas.

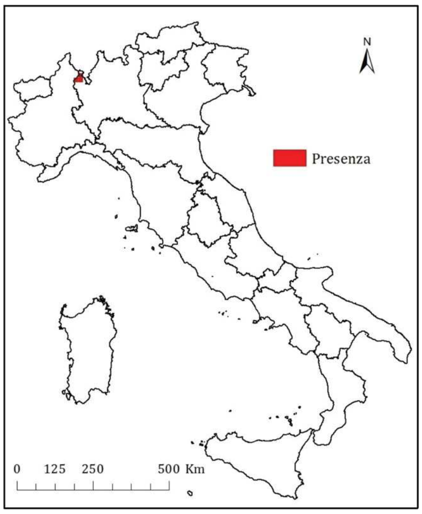


Figure 3. Distribution of *Callosciurus erythraeus* in 10×10 km² cells (June 2019) [Santicchia et al., 2019, ISPRA: Piano nazionale per la gestione dello Scoiattolo di Pallas] [8].

An eradication plan is currently in place with the aim of removing the species from Italian territory, with particular attention given to preventing its spread beyond national borders [8]. Eradication programs are globally recognized as one of the keys to manage biological invasions and there is increasing evidence of their success with 37 successful eradication programs recorded in Europe [17]. Eradicating alien species can support the recovery of biodiversity and should be pursued when prevention, which is the first and most important line of defence, supported by ongoing monitoring and risk assessment, has failed to avoid the invasion [5]. Trapping activities were carried out as part of the European LIFE project EC-SQUARE (LIFE09 NAT/IT/00095), specifically focused on controlling this invasive species. Baiting techniques were designed to selectively target the alien squirrel population, and live traps were used and checked regularly to reduce animal stress. For each captured individual, sex and reproductive status were recorded. Lactating females were released immediately to safeguard dependent offspring, while all other individuals were euthanized using CO_2 inhalation, in accordance with the European Commission (EC) and AVMA animal welfare guidelines [8], [13].

Given that IAS are recognized as potential drivers of pathogen pollution in the ecosystems they colonize [18] it would be reasonable to expect animals recognized as IAS to be a major focus of epidemiological research, especially concerning the potential risks they pose to both human and wildlife health. However, information on the pathogens carried by IAS is still quite limited. This lack of data represents a major barrier to evaluating disease emergence linked to biological invasions, as reliable health-risk assessments require knowledge of the parasites, bacteria, and viruses carried by the invading species [9].

Carrying out a disease risk assessment involves several steps: first identifying the pathogens of concern (the hazards), then assessing the chances that these pathogens are introduced and that the target population is exposed to them, and finally evaluating the potential consequences. Unfortunately, there are gaps in our knowledge at almost every stage of this process, which makes building an effective disease risk model for IAS quite challenging. To properly evaluate these risks it is essential to know which pathogens IAS naturally carry, yet this information remains very limited [5].

The issue becomes even more relevant when dealing with IAS that adapt well to urban environments, where close contact with people can increase the chance of zoonotic transmission. This is especially true for squirrel species, which are one of the few mammals occurring in both natural environments and highly urbanized settings and are often reported to be comfortable around humans [19], which is one of the reasons they were introduced in the first place [7] , raising further concerns about their role in spreading zoonotic diseases.

Given these gaps there is a clear need for studies that investigate which pathogens are carried by invasive species, especially in areas where they are spreading and frequently interact with humans, domestic animals, and wildlife. In this context, this thesis focuses on the Pallas's squirrel (*Callosciurus erythraeus*) and is carried out within a broader research project on the health status of invasive alien species in northern Italy. The study is based on the necropsies of 99 Pallas's squirrel carcasses and aims to provide a screening for pathogens that may be relevant for both human and wildlife health.

CHAPTER 1

FROM BIOLOGY TO IMPACT: UNDERSTANDING THE INVASIVENESS OF THE PALLAS'S SQUIRREL

Callosciurus is a genus of diurnal, arboreal squirrels commonly known as the "beautiful squirrels" [14], a name earned due to their vivid colours and striking appearance. These attractive traits are not only descriptive but also help explain why several species in this genus entered the pet trade, which represents the first reported cause of introduction for the spread of IAS [14], [7], [6].

The visual appeal of exotic animals and plants is often a powerful driver of intentional human transport, and it can easily overshadow ecological risks. Bright colours, unusual shapes or charismatic behaviour can make a species seem harmless, reducing awareness of the potential consequences of escape or release into the wild [5], [20], [21].
Well-known examples illustrate how aesthetic appeal has contributed to biological invasions. The American red-eared slider (*Trachemys scripta elegans*), for instance, became a globally invasive species after the pet trade sold more than 52 million individuals between 1989 and 1997. Its attractiveness and the perceived ease of maintenance directly facilitated its spread worldwide, where it now represents a major threat to native turtle species. This phenomenon is part of a much older pattern, since human fascination with exotic beauty has deep historical roots. As for the case of the water hyacinth (*Pontederia crassipes*): appreciated for its large violet flowers, it was introduced into Europe by Napoleon himself to decorate ponds and gardens. Since then, its accelerated growth has led to the formation of dense mats that currently suffocate native vegetation and alter freshwater ecosystems, causing cascading negative consequences for biodiversity [5].

Squirrels themselves were already kept as pets in early modern Europe, where they appear in treatises on quadrupeds as desirable companions for noblewomen. Their small size, soft fur and lively behaviour made them fashionable animals to keep in private chambers and at court. Given this long history of human interest in squirrels, it is not surprising that several squirrel species have also been moved outside their native range in more recent times. The earliest known introduction of *Callosciurus* occurred in 1935 in Japan, when several Pallas's squirrels escaped from the zoological gardens on Izu-Oshima Island, near Tokyo. In Europe, the first introduction took place in France, between the late 1960s and early 1970s, when a small number of individuals were imported from Asia by a private owner, and their release or escape resulted in the establishment of the initial French population.

The first documented presence of the Pallas's squirrel in Italy dates to 2007, yet the origin of introduction is still unclear. It has been proposed that the Italian population may derive from individuals escaping, or being released, from a private residence in an area with many holiday homes owned by citizens from the Netherlands. This hypothesis is noteworthy considering that a well-known escape event occurred in the Netherlands in 1998 from a local animal trader, and that the invasive population established in Argentina originated from individuals owned by a European family who had purchased them in a pet shop in the Netherlands [14].

The number of recognised species within the genus *Callosciurus* varies among taxonomic references. While Wilson and Reeder [12] list 15 species, more recent taxonomic revisions identify 16 [14]. All species within the genus are native to Southeast Asia [7], [12], [14] and two of them have been introduced outside their native range: Finlayson's squirrels (*C. finlaysonii*) and the Pallas's squirrel (*C. erythraeus*), focus of this study.

1.1 Biology and ecology of the Pallas's squirrel

The Pallas's squirrel (*Callosciurus erythraeus*) is a medium-sized sciuromorph not showing clear sexual dimorphism, as males and females have similar body size and general appearance. Reported body mass ranges from about 309 to 460g, although in Italy adults are usually lighter, with weights between 250 and 320g [8]. In overall measurements the Pallas's squirrel is quite similar to the Eurasian red squirrel (*S. vulgaris*). However, the two species can be easily distinguished by the colour of the belly: *S. vulgaris* typically has a completely white ventral area, while the Pallas's squirrel shows a much wider range of coat colours, with the belly varying from orange or reddish to yellowish tones [22], [8].

Figure 4a. The native red squirrel (*Sciurus vulgaris*) [Renato Olivieri, 2020 - JuzaPhoto.com]

Figure 4b. The invasive Pallas's squirrel (*Callosciurus erythraeus*) on the right [Santicchia et al., 2019, ISPRA: Piano nazionale per la gestione dello Scoiattolo di Pallas] [8].

In its native range the Pallas's squirrel shows a high level of ecological flexibility, thriving in a wide variety of habitats. It can occupy subtropical montane evergreen and broadleaved forests as well as subalpine coniferous and mixed forests at elevations above 3,000 m. This adaptability is also evident where the species has been introduced. In Europe it uses mixed and deciduous woodlands, while in Argentina it persists in fragmented conifer–broadleaved patches. It is also well established in suburban and peri-urban environments in Japan, Belgium and France.

The main factors influencing habitat selection in this species are reported to be high canopy cover, consistent with its arboreal lifestyle, and abundant food resources [12]. Two conditions that are well represented in the northern part of the Varese province (Lombardy) and have favoured the establishment of the species. In this area, the alien population currently covers approximately 9,800 hectares, extending along the mountainous belt from the municipality of Laveno-Mombello in the south to Luino in the north and its density has been estimated at about 7.8 individuals per hectare [8], a value slightly higher than that reported in Taiwan, part of the species' native range.

1.2 Interspecific competition with the Eurasian red squirrel (*Sciurus vulgaris*): traits favoring the success of *C. erythraeus*

In areas where the introduced Pallas's squirrel occurs in syntopy with the native red squirrel, the latter shows a markedly reduced density compared with sites where it is the only squirrel species, with roughly 150 Pallas's squirrels for every single red squirrel [23]. This strong decline seems to result from a combination of habitat displacement and food competition driven by the invasive species. By occupying the best-quality patches and probably competing for cached food [24], the Pallas's squirrels indirectly forces red squirrels into marginal, low-productivity areas where resources are limited, reducing their foraging opportunities and overall fitness.

The competitive advantage of *C. erythraeus* is likely linked to its broad diet and marked dietary plasticity. The species feeds on flowers, fruits, seeds and occasionally insects [12], [22] and is able to exploit alternative food sources when availability is low, such as during winter or periods of high energetic demand. Under these conditions, it may switch to bark stripping, a behaviour widely documented in introduced populations [7], [12]. As a consequence of this combined pressure, red squirrels living in syntopic areas often show lower body mass [24] than individuals living in areas where they are the only species present, and syntopic populations also exhibit a reduced local survival rate [23].

The negative effects on body mass and survival rate could eventually reduce the reproductive output of the red squirrel: as an income breeder, the breeding phenology of *S. vulgaris* is closely linked to the availability of resources within its territory. When these resources are heavily exploited by invasive squirrels, such as the Pallas's squirrel or the grey squirrel, red squirrels reproduce less and may disappear from affected areas within a relatively short time (evidence shows that when S. carolinensis is present, populations of *S. vulgaris* can disappear from the area within a single year) [5], [25].

This process, driven by ecological competition, is one of the mechanisms through which IAS replace native species. According to the competitive exclusion principle, when two species compete for the same limited resources within an ecological niche, the species with a higher fitness will ultimately outcompete the other [26].

Native species that have not evolved in the presence of close competitors may be particularly vulnerable in this context. When exposed to an invasive species, they often lack the behavioural, ecological or physiological adaptations needed to respond effectively. In contrast, invasive species typically display superior competitive abilities, including behavioural plasticity, efficient resource exploitation, lower susceptibility to local pathogens and predators and higher reproductive potential [26].

Interestingly, studies conducted in *C. erythraeus* native range indicate a lower reproductive potential compared to the European red squirrel [12]. The species has a promiscuous mating system and individuals reach sexual maturity at about one year of age, a pattern that is similar to that observed in *Sciurus vulgaris* [12], [8]. Breeding can occur throughout the year, although two main peaks are usually observed: one in spring and one in summer [22], [12] but despite this extended breeding period, native populations show relatively low reproductive output. Reported litter sizes average around 2 young, with a range of 1 to 4, depending on population density, and the number of weaned juveniles is also limited (1.1 on average in a population from Taiwan) [22].

However the situation differs in the introduced range where the invasive species shows higher fecundity, with evidence of up to three litters per year in Japan and Italy [12], [27].

This suggests that the species can adjust its reproductive activity to more favourable environmental conditions, reinforcing its status as a highly adaptable and invasive species [12]. In Italy studies on 40 females recorded an annual production ranging from 0 to 9 offspring per female, with a maximum litter size of 6 (though such cases are infrequent) [27]. This upper value exceeds most reports from both native and other invasive populations, where litters typically consist of two to three young [12], [22].

Although Pallas's squirrel does not appear to directly reduce the fertility of the native red squirrel yet [27], unlike the invasive grey squirrel, which has shown strong negative effects in both England and Italy, its higher reproductive rate provides a clear competitive advantage that may contribute to the progressive displacement of *S. vulgaris*.

The Pallas's squirrel is therefore considered a "good invader" due to its high colonisation potential, with more than a 70% probability that the release of a single pair will lead to the formation of a new population, as seen in Figure 5 [1], [12].

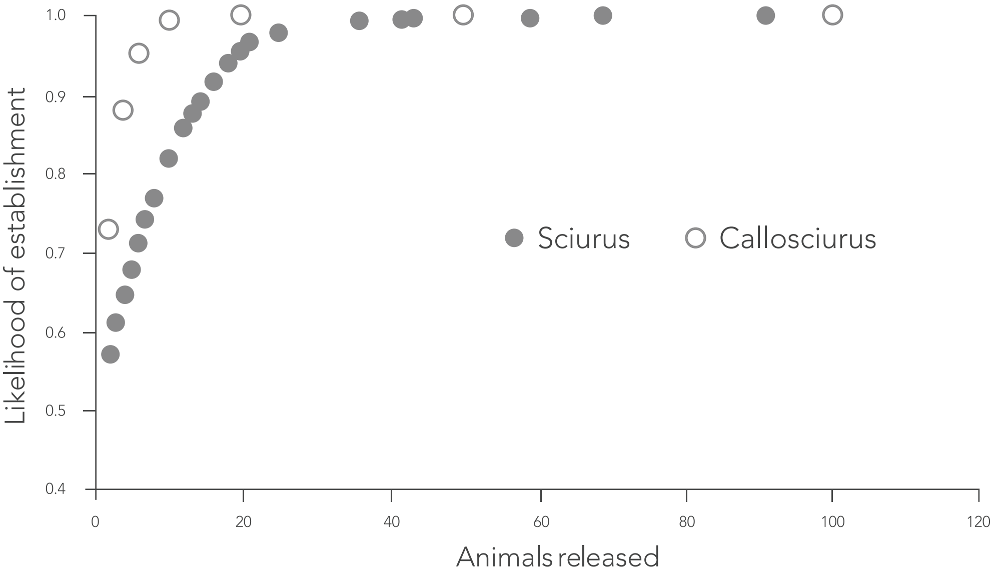


Figure 5. Likelihood of *Sciurus* and *Callosciurus* establishment as a function of the number of animals released (Bertolino et al., 2009) [1]

1.3 Additional ecological and socio-economic impacts of the Pallas's squirrel

Besides its effects on native species, one of the main problems caused by the Pallas's squirrel is its bark-stripping behaviour , [12]. By removing bark, these squirrels can seriously harm trees and timber plantations, making them more prone to fungi and insect infestations, which can ultimately impact the plant and animal species living in those forests [28]. In its native range in Taiwan the species has been identified as the main cause of bark damage, especially on conifer trees [7] and similar issues have been observed in several other introduction areas, including Belgium, France, Japan and Argentina, where *C. erythraeus* has been reported to damage forest plantations, fruit orchards and ornamental trees [7]. However, most of the available data are qualitative and do not allow for a reliable assessment of the quantitative losses linked to the Pallas's squirrel [12].

Bark stripping tends to become more frequent in periods of food shortage, for example during snow cover or in phases of high energetic demand such as the reproductive season. In these moments, squirrels may strip bark to reach the phloem sap, which is rich in sugars.

In Europe, the native red squirrel (*Sciurus vulgaris*) only rarely causes significant damage to forests or arboriculture systems [29].

Some reports from introduced areas mention occasional predation on the eggs of native species by the Pallas's squirrel. Although this behaviour seems rare and currently of limited concern, its impact could grow if squirrel densities continue to rise, with possible consequences for local wildlife [8], [12].

The Pallas's squirrel can also give origin to human-wildlife conflicts. For example, it may build nests in private gardens or close to houses, which can disturb residents. In France, the species was at first appreciated because of its appearance and behaviour, but over time it started to be considered as a pest, even earning the nickname "Korean rat". This change happened mainly because the squirrels can damage different types of infrastructures, such as telephone cables, sprinkler systems and other equipment. As a result, some people have tried to remove them by poisoning or shooting, even though these actions are dangerous and can harm non-target species [7], [12].

The impacts of IAS can take many forms, and it is often difficult to define or quantify them [30]. Some effects, like the loss of habitat or visible damage, can be measured more easily. However, many other impacts are more diffuse, may develop slowly over long periods of time, and are therefore harder to detect or to clearly demonstrate [7].

For instance, a plant or animal introduced today may initially appear harmless, but its population can slowly increase over decades until ecological impacts become evident. A good example is the invasive blue crab (Callinectes sapidus): although it was first recorded in Italy in 1948, its dramatic expansion occurred only in the spring of 2023, causing strong impacts on native species such as oysters, mussels and small fish and consequently on the economic activities that depend on them [5], with an economic loss of 100 million euros between 2023 and

2024 [6]. It is therefore crucial to act promptly as management measures launched only after impacts have become widespread are usually far less effective and much more expensive [6].

Some impacts of biological invasions cannot be quantified economically, particularly those affecting biodiversity [6]. Biological introductions, acting both as direct threats and as synergistic drivers alongside other environmental and anthropogenic pressures, are contributing to unprecedented rates of species decline and extinction [31].

Globally IAS have been identified as the sole cause of 16% of documented species extinctions, and they have contributed to 60% of all recorded global extinctions [6], leading to profound changes in species diversity and community composition across most ecosystems. Such changes in biodiversity can strongly influence how infectious diseases emerge and spread, with major economic, public health, and conservation consequences [31].

CHAPTER 2

ALIEN SQUIRREL-ASSOCIATED PATHOGENS: IMPLICATIONS, POTENTIAL THREATS, AND PARASITE-RELATED INVASION PROCESSES

2.1 Understanding the impact of invasive alien species on disease emergence

The impacts of IAS extend beyond competition, predation, and habitat alteration. An important, yet often underestimated, consequence of biological invasions is their role in the transmission and emergence of infectious diseases [9]. Invasive alien species can contribute to a process known as pathogen pollution, which occurs when human activities such as international trade, global travel, and species translocations, introduce parasites and microorganisms into regions where they were previously absent.
Considering that at least 60% of known human pathogens are zoonotic, with approximately 70% originating from wildlife reservoirs [30], [32] and infectious diseases are among the top five drivers of global species extinctions [33], IAS and their associated pathogens can represent a significant threat to both biodiversity and human health [32].

Global trade and climate change are rapidly altering ecosystems worldwide, increasing opportunities for the introduction and spread of IAS [34], [35], which have been demonstrated to play a significant role in the transmission and emergence of infectious diseases, influencing ecosystem health through the pathogens they introduce, acquire, or leave behind [18].
Evidence accumulated over the past two decades indicates a positive association between the presence of IAS and an increase in zoonotic disease emergence events [10], [36], a relationship which remains evident even when major anthropogenic drivers, such as climate change and land-use change, are taken into account (Figure 6) [35].

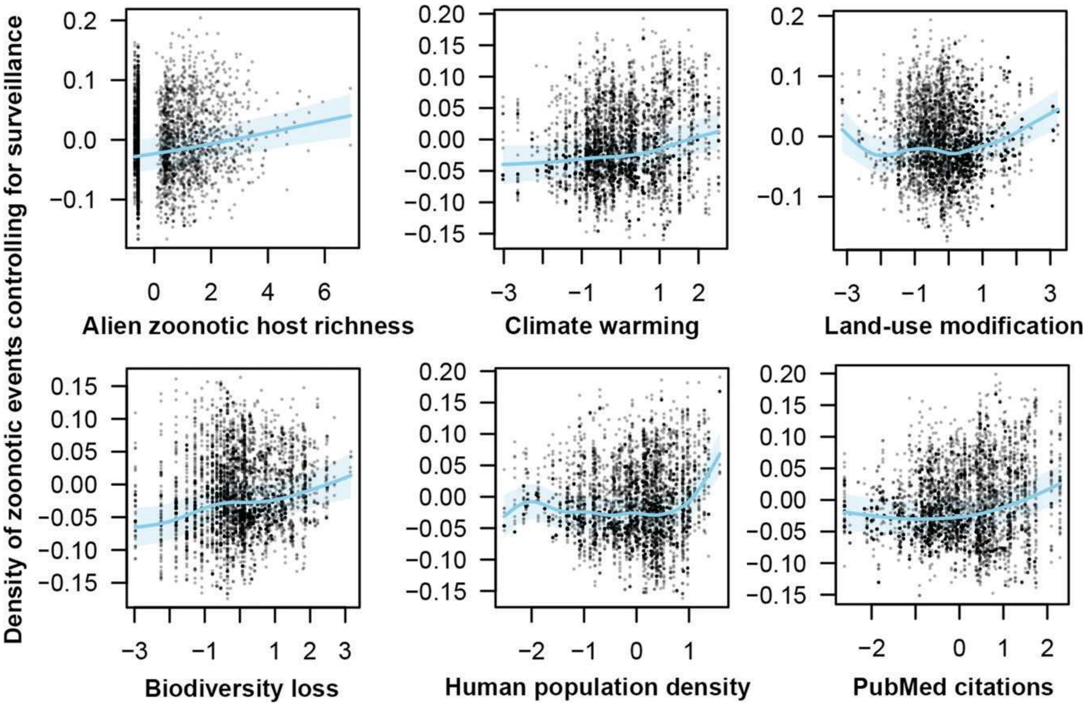


Figure 6. Association between selected drivers and zoonotic disease emergence [Zhang et al., 2022]
The x-axis shows how much each variable differs from the global average in standard deviations, while the y-axis shows whether a region has more or fewer zoonotic events than expected after correcting for surveillance effort. The blue line represents the overall trend estimated by the model, with shaded areas indicating 95% confidence intervals [35]

Quantitative analyses have estimated an average of approximately 5.9 zoonotic pathogens per alien host species and have identified a marked increase in zoonotic emergence events around 1962, shortly after a major rise in alien host introductions [35].

The close proximity of the breakpoints for species introductions and disease events, suggests a strong temporal link between the global movement of animals and the emergence of zoonotic diseases. Overall, the data in Figure 7 show that the middle of the 20th century was an important period that changed the way biological risks spread around the world.

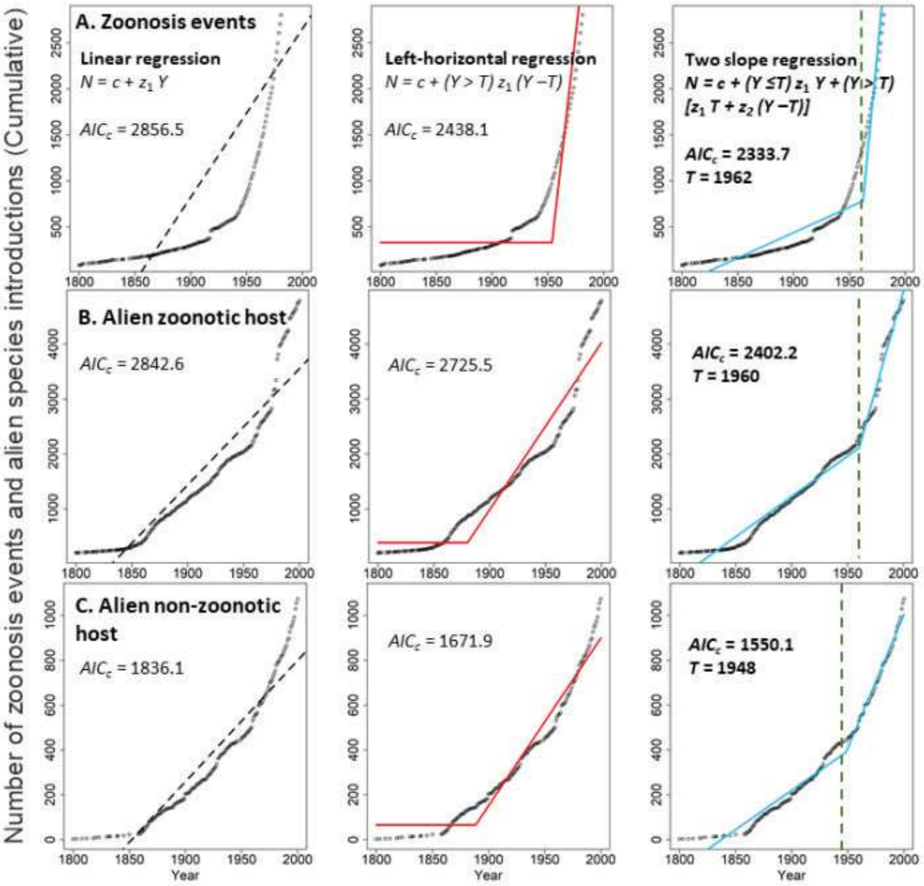


Figure 7. historical trajectory of zoonotic events and alien species introductions between 1800 and 2000 using three different statistical models [Zhang et al., 2022]

In all categories (A, B, and C), the Two-slope regression (right column) is identified as the best fit. The analysis reveals a significant "breakpoint" (marked by the green dashed line) where the rate of these events shifted from slow growth to rapid acceleration. Specifically, the introduction of non-zoonotic species began to surge around 1948, followed closely by a surge in zoonotic host introductions in 1960 and actual zoonotic disease events in 1962 [35]

A substantial proportion of IAS also have significant impacts on wildlife health. In fact, approximately one quarter of the species included among the 100 of the World's Worst Invasive Alien Species identified by the International Union for Conservation of Nature (IUCN) are associated with environmental impacts related to diseases affecting wildlife [37]. A well-known example is the chytrid fungus (*Batrachochytrium dendrobatidis*), which has been strongly associated with global amphibian population declines and multiple species extinctions [9]. Amphibians are currently considered the most threatened vertebrate group, according to assessments by the IUCN.

2.1.1 *Pathways of pathogen transmission in biological invasions*

Depending on whether pathogens originate from the species' native range, are acquired during transit through intermediate regions, or are picked up after establishment in the new environment, invasive hosts can interact with local species in different ways (Figure 8) [38].

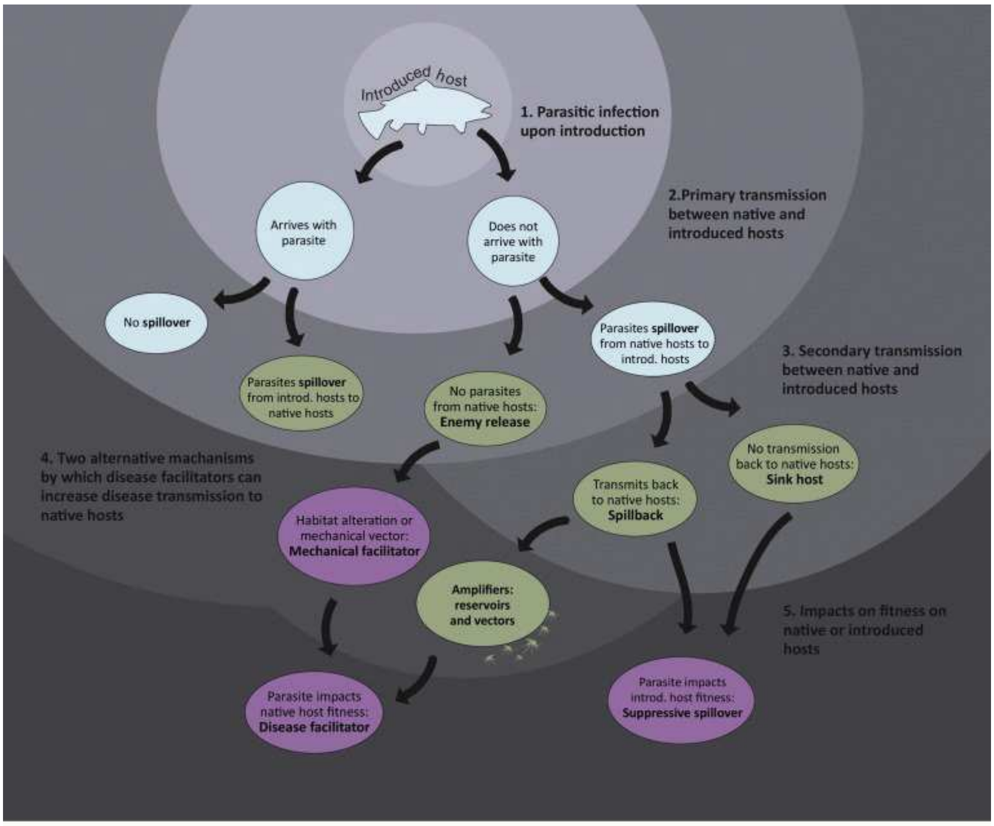


Figure 8. Potential Pathways for Any Given Introduced-Host Parasite System. [Chalkowski, Lepczyk, Zohdy, 2018]

Because an introduced host may carry multiple parasites at the same time, any of the following possibilities can coexist for a given introduced host species. (a) The introduced host brings a parasite from its native range to its introduced range, where the given parasite can be transmitted to native species via host-switching or spillover, or not. (b) The introduced species may arrive to the introduced range without a parasite where a number of interactions with parasites of native hosts is possible. (c) The introduced species does not obtain parasites of native hosts, thereby allowing for enemy release to occur. (d) The introduced species does not become infected with parasites of native hosts, but aids parasite spread as a mechanical facilitator through habitat alteration or as a mechanical vector. (e) Parasites of native hosts are transmitted to the introduced species where the introduced host can (f) act as a sink host that does not transmit parasites back to native hosts, (g) suffer fitness consequences via suppressive spillover, or (h) transmit parasites back into native hosts via spillback. (i) Introduced species can facilitate parasites of native hosts via classic roles of spillback as amplifiers or vectors, and as mechanical facilitators [38].

When an IAS carrying a pathogen from its native range is introduced into a new ecosystem, it may come into contact with native hosts, facilitating the transmission of a novel pathogen through pathogen spillover: the process by which a pathogen maintained in a reservoir host is transmitted to a new host species that has not previously been exposed to it [9], [10], [38], [34]. Such pathogens can spread rapidly because native hosts often lack prior exposure and effective immunological defenses, making them highly susceptible to infection and allowing invasive pathogens to amplify and cause severe outbreaks [32].

A prominent example in Europe is the introduction of the North American raccoon (*Procyon lotor*), which acts as the definitive host for the nematode Baylisascaris procyonis. This parasite can cause larva migrans syndromes in humans, potentially leading to severe central nervous system disease [18].

Conversely, IAS may become infected with local pathogens already present in the invaded range, leading to increased opportunities to infect native species through pathogen spillback. In this scenario, the invasive species acts as a new reservoir, increasing the amount of the pathogen in the environment [9], [10], [38], [34] .

This is exemplified by the Australian brushtail possum (*Trichosurus vulpecula*) in New Zealand. Following its introduction, the possum became infected with bovine tuberculosis (*Mycobacterium bovis*), eventually becoming a primary maintenance host that amplifies the disease and facilitates its transmission back to livestock and native wildlife [18].

These two mechanisms represent direct pathways through which IAS can increase the risk of disease transmission and are strongly supported by real-world example. However, IAS may also enhance disease spread through indirect mechanisms [38]. In some cases, invasive species are not infected by the pathogen themselves but instead alter local ecosystems and the behavior of native hosts or vectors, thereby modifying existing disease dynamics [9], facilitating the diffusion of pathogens [38]. Disease facilitation refers to the process by which an introduced species increases infection by pathogens already present in native hosts, either through pathogen amplification (by influencing vector populations) or through mechanical habitat alteration, that improves conditions for pathogens transmission [38]. A clear example is provided by the invasion of the Burmese python (*Python molurus bivittatus*) in the Florida Everglades. The python has caused a drastic decline in large mammal populations, leading local mosquito vectors to shift their feeding behavior toward smaller mammals, such as the hispid cotton rat (*Sigmodon hispidus*), the primary reservoir of the Everglades virus. This shift increases viral prevalence in rat populations and elevates the risk of transmission to humans, particularly given the tendency of these rodents to thrive in anthropogenic environments [18]. In this case, the invasive Burmese python acts as a disease facilitator through its predation-driven alteration of the host community.

The role of invasive alien species in disease transmission is therefore not limited to whether they directly host a pathogen, but also includes the ecological effects they exert in the invaded area, as a consequence of their invasiveness.
Empirical evidence shows that native populations host approximately twice as many parasites species as invasive populations [39], a pattern described by the Enemy Release Hypothesis (ERH), which proposes that alien species flourish in new environments because they escape the natural predators and pathogens that regulate their populations in their native range [39], [40]. Freed from these natural regulators, invasive alien species can allocate more energy to growth and reproduction, facilitating their spread and increasing their ecological impact within the invaded ecosystem. This population expansion may mechanically facilitate parasite transmission, as previously explained, by altering habitats and host–vector interactions [38]. Another example of this is provided by invasive wild boars (*Sus scrofa*), whose rooting behavior creates extensive soil disturbance and wallows. In Hawaii, these wallows, together with tree fern hollows damaged by wild boars, frequently accumulate standing water, creating suitable breeding sites for invasive mosquito species. These mosquitoes are competent vectors of the avian malaria parasite Plasmodium relictum. By increasing the availability of mosquito breeding habitats, wild boars indirectly facilitate the transmission of avian malaria, which is considered one of the main drivers of population declines in critically endangered Hawaiian

forest birds [38]. Furthermore, many IAS are well adapted to human-modified landscapes, which increases contact with humans and domestic animals and enhances opportunities for pathogen transmission [8]. Although invasive populations often exhibit a lower pathogen richness then in the area of origin [9], [35], they may exhibit a higher prevalence of infection for specific zoonotic pathogens [10].

In a systematic review of 234 papers analyzing the relation between the transmission of zoonotic pathogens and invasive alien species, a subset of comparative studies identified 65 host–pathogen interactions between invasive alien and native species. Across these comparisons, the pathogen prevalence within sampled invasive alien species was found to be "lower than in the native host in 15 studies, equivalent to the native host in 14 studies, and higher than the native host in 33 studies" (Roy et al.) [10]. The potential impact IAS represent in the transmission of zoonotic pathogens is linked to a wide range of ecological, environmental and social processes as their ability to thrive in human-dominated environments, where high population densities facilitate pathogen transmission. Synanthropic species, such as rats, represent well-documented examples of this phenomenon [10].

Despite the growing evidence that invasive alien species play an important role in the emergence and spread of infectious diseases, risk assessment approaches in invasion biology have traditionally focused mainly on economic and environmental impacts, often giving limited attention to risks for public and animal health [9], [34]. Information on pathogens associated with IAS is still fragmented and not systematically integrated into major international databases, such as the European Alien Species Information Network (EASIN) which provides information on the distribution, pathways and impacts of alien species in Europe, and the Global Invasive Species Database (GISD), a worldwide reference platform documenting invasive species, their impacts, and management responses. This lack of integration hampers the development of comprehensive and reliable disease risk assessments [9].

Having outlined the general role of invasive alien species in disease emergence and pathogen transmission, the following chapters focus on the specific case of the Pallas's squirrel (*Callosciurus erythraeus*).

2.2 Pathogen risks related with the invasive Pallas's squirrels

As discussed in section 2.1, IAS are increasingly recognized as significant drivers in the spread of infectious diseases, particularly in the case of mammals [10], [34], with rodents as the most species-rich zoonotic mammalian hosts [10], [41] (as seen in Figure 9). This growing awareness has led many researchers to emphasize the importance of predicting and managing the health risks associated with biological invasions through the development of specific risk assessment tools, horizon scanning approaches and standardized health evaluation procedures, including necropsy and targeted pathogen screening [9], [10], [18], [34].

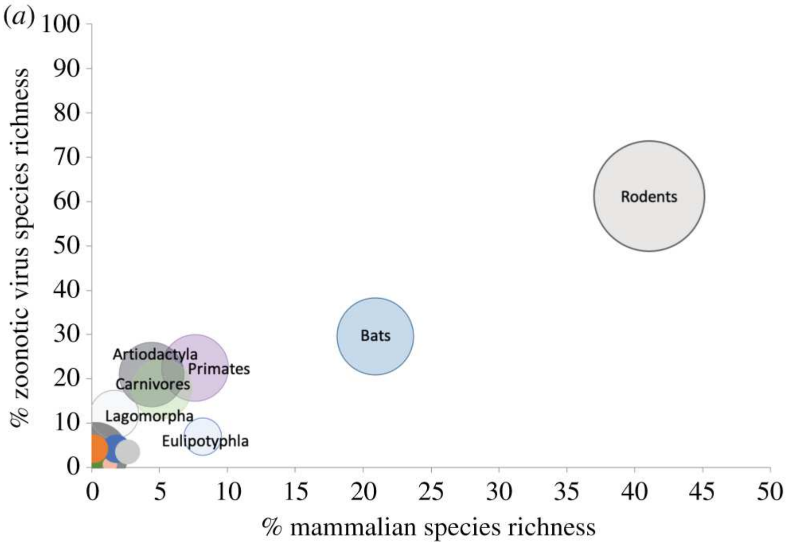


Figure 9. Zoonotic virus richness in relation to species richness among wild mammalian orders, with circle size representing the proportion of zoonotic viruses recorded in each order relative to the total across all mammals [C. K. Johnson et al., 2020] [41]

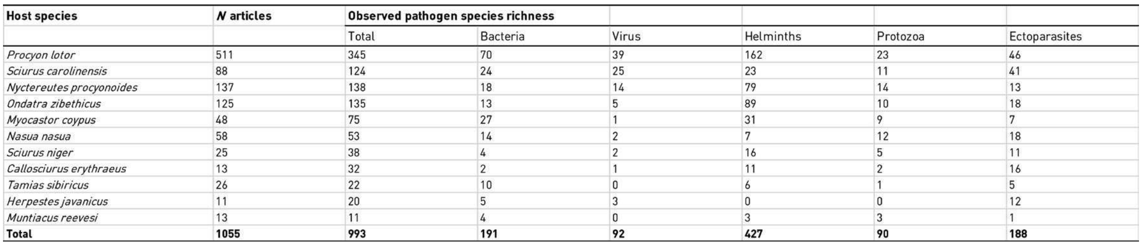
Evaluating the disease risk associated with invasive alien species requires a broad understanding of the host–pathogen relationships and their interactions with the new environment. This includes considering their role in the maintenance and spread of infectious agents and the possible implications for wildlife, domestic animals, and public health. As a starting point it is necessary to identify the hazards of interest, namely the pathogens that are associated with the species under natural conditions [9], [37].

In the case of *C. erythraeus*, current knowledge is still scarce, particularly with regard to the pathogens associated with the species in its native range [9], [12], [42], [43]. Most available data concern macroparasites in introduced areas [9], [13], [42] whereas information on microparasites remains poor.

According to the systematic review by Chinchio et al. (2022) [9], which examined over 1,000 articles on 11 mammalian IAS of Union concern in Europe, *C. erythraeus* is among the species with the most limited information on its pathogen profile. Only 13 articles met the eligibility criteria for this species (primary research, written in English, available online and based on natural infections), and the available data were insufficient to estimate pooled prevalence for any of the relevant pathogens associated with the squirrel. This means that while these pathogens have been reported, there is not enough scientific evidence to determine how common they are in the wild populations [9].

The review identified 32 infectious agents associated with *C. erythraeus* (Table 1), including 16 ectoparasites, 11 helminths, 2 bacteria, 2 protozoa, and 1 virus [9]. The data is heavily skewed toward macroparasites, which represent the 84% of the recorded pathogens (27 of 32), highlighting a clear research effort bias: most of the available information concerns pathogens that are visually detectable, while micro-pathogens remain largely uninvestigated.

Table 1. Number of articles, total observed pathogen species richness, and observed pathogen species richness per pathogen taxon [Chinchio, Romeo, Crotta, and Ferrari, 2022] [9].



Host species	***N* articles**	**Observed pathogen species richness**					
		Total	Bacteria	Virus	Helminths	Protozoa	Ectoparasites
Procyon lotor	511	345	70	39	162	23	46
Sciurus carolinensis	88	124	24	25	23	11	41
Nyctereutes procyonoides	137	138	18	14	79	14	13
Ondatra zibethicus	125	135	13	5	89	10	18
Myocastor coypus	48	75	27	1	31	9	7
Nasua nasua	58	53	14	2	7	12	18
Sciurus niger	25	38	4	2	16	5	11
Callosciurus erythraeus	13	32	2	1	11	2	16
Tamias sibiricus	26	22	10	0	6	1	5
Herpestes javanicus	11	20	5	3	0	0	12
Muntiacus reevesi	13	11	4	0	3	3	1
Total	**1055**	**993**	**191**	**92**	**427**	**90**	**188**

Of these 32 infectious agents identified in *C. erythraeus*, only two pathogen species were recognized as having public health and wildlife health significance, according to European legislation and international health organizations: *Leptospira interrogans* and *Cryptosporidium spp.*

Leptospira interrogans, was detected in an introduced population of *C. erythraeus* in Argentina, where 13 of 34 squirrels examined (38% prevalence) tested positive [44], and has also been reported in the species' native range: a study in Laos found the bacterium in 12 of 73 Pallas's squirrels sold for human consumption [45].

The second relevant agent, *Cryptosporidium spp.* has been reported in introduced populations in Japan and Italy [46], [47]. In addition, *Cryptosporidium spp.* have been detected in captive individuals of *C. erythraeus* sold as pets in China [48].

Despite these two infectious agents being present in both native and introduced populations of Pallas's squirrels, evidence suggests that in the invaded range the pathogens were most likely acquired locally rather than introduced from the native range.

For what concern *Leptospira interrogans*, this hypothesis is supported by the fact that the entire Argentinean invasion originated from only 10 individuals bought from a pet shop in the Netherlands in 1970, with genetic analysis confirming a founder effect, showing that all Argentinean populations likely stem from this single, small introduction. In addition, the serovars found in the squirrels (*Canicola* and *Copenhagen*) are frequently found in Argentinean peridomestic animals like dogs and rats. Because Pallas's squirrels in Argentina inhabit urban and rural areas in close contact with these animals, they likely entered the local epidemiological cycle after their release [44]. Furthermore, studies on other parasite groups, such as gastrointestinal helminths, in Argentine populations of Pallas' squirrels indicate a loss of their native Asian parasites and the acquisition of novel parasites from local Argentine rodents [43].

Regarding *Cryptosporidium spp.*, a study conducted in China, within the native range of Pallas' squirrel, identified *C. parvum, C. wrairi*, and *rat genotype II.* These taxa have not been reported in invasive populations in Italy or Japan [46], [47], [48].

2.3 Pathogen profile of Pallas's squirrels in Italy: insights from literature

In line with findings from other introduced populations worldwide, Pallas's squirrels in Europe appear to harbour a limited range of pathogens, most of which seem to have been acquired locally rather than introduced from the native range [13], [14], [42].

Similar findings were reported in studies investigating pathogens in Italian populations of Pallas's squirrel.

Mazzamuto et al. [13] examined 161 Pallas's squirrels from Italy's only known population, in Varese province, and described the invasive colony as hosting a relatively poor parasite community. With only one exception (the nematode *Strongyloides callosciureus*), all identified parasite species were native to Europe, indicating that they were acquired locally after the establishment of the squirrels in Italy [13].

2.3.1 Ectoparasites

In the 135 squirrels hosting ectoparasites, the most prevalent species was the flea *Ceratophyllus* (*Monopsyllus*) s*ciurorum sciurorum*, detected in 50% of the individuals. This flea is a specific parasite of the native red squirrel (*Sciurus vulgaris*). The high prevalence observed in the invasive population is likely the result of ecological overlap with the native species, including habitat sharing and the use of the same nests. This interaction creates favorable conditions for parasite exchange and may result in spill-back dynamics [13].

The second most common ectoparasite was the tick Ixodes ricinus, with a prevalence of 47%. Unlike C. sciurorum, this tick is a generalist species whose distribution depends mainly on environmental factors and the presence of suitable hosts, such as roe deer (*Capreolus capreolus*), rather than on squirrels specifically. While *I. ricinus* has been reported on both native and introduced squirrels in France, it had interestingly not been recorded on *S. vulgaris* or *S. carolinensis* in Italian study sites previously, likely due to differing ecological factors such as local ungulate density [13]. The heavy infestation of Pallas's squirrels by these ticks introduces a potential risk to human health, as these introduced sciurids could influence local transmission dynamics of tick-borne pathogens, including *Borrelia burgdorferi*, the causative agent of Lyme borreliosis (LB) [14]. Furthermore, Pallas's squirrels exhibit a high nymph-to-larvae ratio of approximately 2:1, which is likely attributed to their arboreal behavior [13]. This observation is ecologically significant because, whereas tick larvae are generally pathogen-free, nymphs have typically completed a prior blood meal. Consequently, they are more likely to carry and transmit infectious agents to both the vertebrate community and humans.

While the risk of spillover from the invasive squirrel to the native species has not been observed yet it remains a possibility, along with the potential alteration of tick-borne pathogen distribution as the invasive squirrel may acts as a new, significant host for local disease-carrying ticks [49].

2.3.2 Endoparasites

Endoparasites were considerably less common than ectoparasites. Of the 73 squirrels examined for gastrointestinal helminths, only 12 individuals were infected, and all parasite species showed a prevalence below 10% (Table 2). Most nematodes identified in the gastrointestinal tract were acquired locally. For example, *Trypanoxyuris (Rodentoxyuris) sciuri*, a specific parasite of the native red squirrel, confirms the occurrence of spillover from native hosts to the invasive species. Although this parasite can complete its life cycle in Pallas's squirrels, its low prevalence suggests that spillover events are relatively rare and that the parasite may not yet be fully adapted to this new host. Consequently, the risk of spillback to native red squirrels is currently considered low. Another species, *Trichuris muris*, is commonly associated with other rodents, such as mice and voles, suggesting cross-species transmission within the local rodent community [13].

Only one nematode species, *Strongyloides callosciureus*, appears to have been co-introduced with *C. erythraeus* into Italy. This parasite, a dominant nematode within the gastrointestinal helminth fauna of Pallas's squirrels, has been previously reported in introduced populations in Japan, with a prevalence of 73%, likely co-introduced with the host species [50]. Its prevalence in Italy is extremely low (1%) and it has not been repored in native squirrels. This low prevalence may be linked to founder effects and reduced genetic variability in the Italian squirrel population, potentially limiting parasite establishment and transmission [13]. Some studies suggest it may have relatively broad host potential among rodents [54], but confirmed records are still mostly from sciurids.

Table 2. Prevalence and intensity (± SE) of helminths and arthropods in Italian *C. erythraeus*. Italics denote low-frequency infections (n<10) [M. V. Mazzamuto et al., 2016] [13].

Macroparasite species	**Prevalence (%)**	**Intensity ± SE**
Helminths (total number of examined hosts = 73)		
Trypanoxyuris (R.) sciuri	5	*1, 1, 1, 6*
Trichuris muris	4	*1, 1, 1*
Strongyloides callosciureus	1	*1*
Strongyloides sp.	1	*1*
Capillariinae	1	*1*
Spiruridae	1	*1*
Arthropods (total number of examined hosts = 135)		
Ceratophyllus (M.) s. sciurorum	50	1.0 ± 0.1
Ixodes (I.) ricinus	47	3.0 ± 0.7
Trombiculidae	7	–
Ctenophthalmus (C.) agyrtes sardiniensis	1	*1*
Ctenophthalmus sp.	1	*1*

2.3.3 Microparasite

For microparasite, data are very scarce. A recent study in Italy looked at the presence of coccidia (genus *Eimeria*) in both native and invasive squirrels and found that *Callosciurus erythraeus* has a very different parasite profile compared to other squirrel species. The study identified three types of *Eimeria* (E1, E2, and E3), which were distributed differently among the hosts. Native red squirrels and invasive grey squirrels had high infection rates, around 95–96%, while Pallas's squirrels showed a much lower prevalence of only 4.1%.

Morphological examination showed that the E2 type was present in all three squirrel species, while E1 was only found in red squirrels and E3 in red and grey squirrels. However, Pallas's squirrels shed very few oocysts, which meant there was not enough DNA for molecular analysis. Therefore, it is still unclear whether the parasite found in Pallas's squirrels is specific to them or came from the red squirrels. In contrast, DNA analysis of red and grey squirrels clearly showed that they carry separate, host-specific coccidia [51].

Another recent study investigated *Cryptosporidium spp.* infection in native and invasive tree squirrels in northern Italy. *Cryptosporidium* is a genus of protozoan parasites that infect epithelial cells of the gastrointestinal tract in all classes of vertebrates, causing cryptosporidiosis, a diarrheal disease [46]. Transmission occurs mainly via the fecal–oral route, and oocysts are environmentally resistant, making the parasite particularly relevant in waterborne contamination.

The study analyzed fecal samples of a total of 357 individuals (123 *Sciurus vulgaris*, 162 Sciurus carolinensis, and 72 *Callosciurus erythraeus*) across 17 locations in Northern Italy revealing that, despite overlapping ranges (with no site hosting all three species simultaneously), each species harboured distinct *Cryptosporidium* taxa (Table 3) [46].

i. Native red squirrels were exclusively infected with the *Cryptosporidium* ferret genotype. This genotype was absent in both invasive species, suggesting a lack of horizontal transmission from native to alien populations in the study area. Interestingly, the probability of infection in red squirrels was significantly affected by habitat: no infected animals were recorded in lowland woods, whereas 20.3% of those in mountain conifer forests tested positive. This distribution suggests that the circulation of the ferret genotype may be linked to extrinsic ecological factors, such as the presence of other host species, likely mustelid, that are absent in lowland habitats [46].

ii. Grey squirrels were found to harbor a diverse array of non-native pathogens: *C. ubiquitum*, the skunk genotype, and chipmunk genotype I. The detection of *C. ubiquitum* subtype XIIb is particularly noteworthy as this subtype is common in North American rodents but previously unrecorded in Europe. This finding suggests that grey squirrels may have acted as the primary introduction pathway from North America [46].

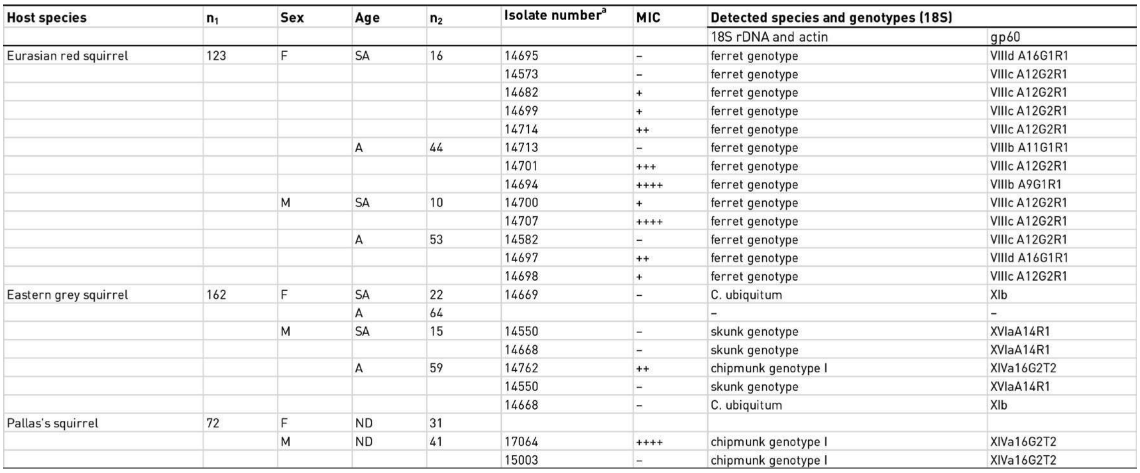
iii. On the contrary, the role of *Callosciurus erythraeus* appears to be that of a secondary host. This species was found to carry the chipmunk genotype I (subtype XIVa), identical to the strains found in grey squirrels. Given that Pallas's squirrels and grey squirrels did not cohabit the specific sampling sites (although unobserved ecological contact cannot be excluded) , the acquisition of this alien parasite likely occurred through environmental mediation [46]. The persistence of oocysts in water or soil suggests that Pallas's squirrels may have become infected via substrates previously contaminated by grey squirrels. This reinforces the hypothesis that invasive sciurids can maintain and circulate introduced pathogens, representing a potential sanitary risk [52].

The detection of *Cryptosporidium* chipmunk genotype I in both grey and Pallas's squirrels, but not in red squirrels in this specific study, suggests that transmission to native species had not occurred in those particular populations. However, previous research in Northern Italy had already reported this genotype in Italian red squirrels, demonstrating that interspecific transmission to native populations could be possible [52].

An important finding is that none of the examined squirrels (red, grey, or Pallas's) showed clinical signs such as diarrhea, even when heavily infected. This suggests that these species can act as asymptomatic carriers, potentially contributing to environmental contamination and parasite spread without displaying disease [46].

Finally, the presence of these genotypes raises zoonotic concerns. The three taxa identified in invasive squirrels (*C. ubiquitum*, the skunk genotype, and chipmunk genotype I) are all known to infect humans. Chipmunk genotype I, introduced to Europe, is increasingly associated with human cryptosporidiosis in the United States. Although human cases in Europe remain rare (with only a few reported in Sweden and France), the presence of this genotype in wildlife represents a potential public health concern [46], [49].

Table 3. The distribution of *Cryptosporidium spp.* in Eurasian red squirrels (*Sciurus vulgaris*), eastern grey squirrels (*Sciurus carolinensis*), and Pallas's squirrels (*Callosciurus erythraeus*) [J. Prediger et al., 2021] [46]



Host species	n_1	Sex	Age	n_2	Isolate number[a]	MIC	Detected species and genotypes (18S)	
							18S rDNA and actin	gp60
Eurasian red squirrel	123	F	SA	16	14695	–	ferret genotype	VIIId A16G1R1
					14573	–	ferret genotype	VIIIc A12G2R1
					14682	+	ferret genotype	VIIIc A12G2R1
					14699	+	ferret genotype	VIIIc A12G2R1
					14714	++	ferret genotype	VIIIc A12G2R1
			A	44	14713	–	ferret genotype	VIIIb A11G1R1
					14701	+++	ferret genotype	VIIIc A12G2R1
					14694	++++	ferret genotype	VIIIb A9G1R1
		M	SA	10	14700	+	ferret genotype	VIIIc A12G2R1
					14707	++++	ferret genotype	VIIIc A12G2R1
			A	53	14582	–	ferret genotype	VIIIc A12G2R1
					14697	++	ferret genotype	VIIId A16G1R1
					14698	+	ferret genotype	VIIIc A12G2R1
Eastern grey squirrel	162	F	SA	22	14669	–	C. ubiquitum	XIb
			A	64			–	–
		M	SA	15	14550	–	skunk genotype	XVIaA14R1
					14668	–	skunk genotype	XVIaA14R1
			A	59	14762	++	chipmunk genotype I	XIVa16G2T2
					14550	–	skunk genotype	XVIaA14R1
					14668	–	C. ubiquitum	XIb
Pallas's squirrel	72	F	ND	31				
		M	ND	41	17064	++++	chipmunk genotype I	XIVa16G2T2
					15003	–	chipmunk genotype I	XIVa16G2T2

Age: SA (Subadult), A (Adult), or ND (Not Determined). Subgroup Sample: specific number of individuals that fall into the combined sex and age category. Isolated numbera: PCR positive samples. MIC: Microscopic Examination, – microscopically negative; + mild infection; ++ medium infection; +++ severeinfection; ++++ intense infection.

Regarding viral pathogens, recent concerns arose following the discovery of the variegated squirrel bornavirus 1 (VSBV-1), a pathogen associated with cases of fatal encephalitis in animal caretakers. Because VSBV-1 was detected in captive squirrel populations of a closely related Asiatic species (*Callosciurus prevostii*) located in Germany, the Netherlands, and Croatia, the wild introduced population of *C. erythraeus* in Italy was subsequently screened for the virus.

Fortunately, the Italian population tested entirely negative, suggesting that *C. erythraeus* is not a reservoir host for this dangerous zoonotic bornavirus. However, this viral screening led to the discovery of three novel viruses circulating within the Italian *C. erythraeus* population: one polyomavirus (*Callosciurus erythraeus* polyomavirus 1) and two herpesviruses (*Callosciurus erythraeus* betaherpesvirus 1 and gammaherpesvirus 1) [53]. Because these viruses were detected exclusively in this species, it is highly likely they are species-specific and that Pallas's squirrels are their original natural hosts, having introduced these novel microparasites to the invaded environment via the exotic pet trade [14].

Table 4. Summary of pathogens identified in the invasive Pallas's squirrel (*Callosciurus erythraeus*) population in Italy. The table outlines prevalence rates, infection origins, and associated epidemiological risks based on the current literature

Virus	Pathogen species	Sample Size (n)	Prevalence (%)	Origin of Infection	Associated Risk	Study
Ectoparasite	***Ceratophyllus sciurorum sciurorum***	135	50%	Acquired (from native red squirrels)	Spillover to invasive Pallas's squirrels; potential future spillback to native squirrels	Mazzamuto et al. (2016)
	Ixodes ricinus	135	47%	Acquired (environmental/generalist)	Spillover to native squirrels; possible human health risk via tick-borne pathogens	Mazzamuto et al. (2016)
Endoparasites	***Trypanoxyuris sciuri***	73	5%	Acquired from native squirrels	Spillover from red squirrels to Pallas's squirrel rare	Mazzamuto et al. (2016)
	Strongyloides callosciureus	73	1%	co-introduced	S. callosciureus low prevalence but potential future risk	Mazzamuto et al. (2016)
Microparasite (protozoan)	**Coccidia (*Eimeria* spp. E2)**	170 fecal sample/159 squirrels	4.1%	Host-specific; unclear if introduced or acquired	Low immediate risk; host-specific parasites; unlikely spillover	Hofmannová et al. (2016)
	Cryptosporidium spp. (chipmunk genotype I)	72	2,8%	Acquired (environmental contamination), likely introduce with grey squirrel	Zoonotic potential; environmental contamination; possible risk to humans and native squirrels	Prediger et al. (2017)
Virus	**Polyomavirus**	35	42,9%	Likely co-introduced with Pallas's squirrels	Unknown pathogenicity; likely species-specific; possible emerging risk	Schulze et al. (2020)
	Herpesviruses	35	65,7%	Likely co-introduced with Pallas's squirrels	Unknown pathogenicity; likely species-specific; possible emerging risk	Schulze et al. (2020)

CHAPTER 3
MATERIAL AND METHODS

As presented in the previous chapter, to date, research in the introduced ranges of *C. erythraeus* has generally reported an overall poor pathogen species richness, supporting the enemy release hypothesis [13], [42], [43], [51]. However, a significant lack of data may bias these findings, particularly concerning microparasites, which are recognized as major drivers of wildlife epidemics. Their specific life-history traits, such as direct life cycles and the lack of intermediate stages, facilitate their emergence and allow them to persist in a wide range of environmental conditions [36].

In this context, where an increased effort in the screening and health assessment of IAS could improve health surveillance and the prevention of disease emergence, necropsies of 99 Pallas's squirrel from Northern Italy were carried out to provide a screening for pathogens that may be relevant for both human and wildlife health.

A total of 99 *Callosciurus erythraeus* were captured in Valcuvia (Varese province, northern Italy) - which hosts the only known Italian population of this species – between January 10th and February 27th, 2025. (Complete data are provided in APPENDIX A).

3.1 Euthanasia suppression technique

Squirrels were captured using live traps (model 202, Tomahawk Live Trap Co., Wisconsin, USA). Traps were set in the morning, baited with apple and hazelnuts, and checked twice daily in order to minimize animal stress. For each captured individual, sex and reproductive status were recorded. Lactating females, identified by enlarged nipples and milk secretion upon gentle pressure, were immediately released to ensure the welfare of dependent offspring. All males and non-reproductive females were euthanized by CO_2 inhalation in accordance with European Commission and American Veterinary Medical Association (AVMA) guidelines.

Each carcass was placed in a sealed plastic bag immediately after death and stored at –20 °C until further examination. Only adult individuals were included in the present study.

Body mass and right forelimb length were recorded, as these measurements are important for comparisons with native species and for assessing potential interspecific competition.

3.2 Post-mortem technique

Each carcass was subjected to a complete post-mortem examination following standard small mammal necropsy protocols, adapted for sciurid species. Prior to necropsy, each specimen was weighed to the nearest 0.5 g using a precision balance. The external examination included assessment of body condition score, nutritional status evaluated through palpation of subcutaneous and visceral fat deposits. The entire body surface was systematically inspected for the presence of ectoparasites (ticks, fleas, mites, and lice), traumatic lesions, integument abnormalities, swellings, discharges, and congenital malformations. The oral cavity, eyes, ears, and anogenital region were individually examined. When ectoparasites were detected, they were collected using fine forceps, preserved in 70% ethanol, and stored in individually labelled vials for subsequent taxonomic identification.

Following external examination, the carcass was placed in dorsal recumbency. A midline incision was made through the skin from the mandibular symphysis to the pubic symphysis, and the skin was reflected bilaterally to expose the underlying musculature and body cavities. The abdominal cavity was opened by a ventral midline incision through the abdominal wall, and the thoracic cavity was accessed by bilateral parasternal incisions through the rib cage using scissors, followed by removal of the sternum. Upon opening each body cavity, the presence of abnormal fluid accumulations, including ascites, hydrothorax, haemothorax, or pericardial effusion, was recorded and fluid samples were collected when present. The overall disposition and appearance of the visceral organs were assessed in situ prior to their individual removal.

Each organ was then systematically removed, weighed, and examined macroscopically. The heart was examined externally and sectioned to assess myocardial thickness, chamber dimensions, valvular integrity, and the presence of endocardial or epicardial lesions. The lungs were assessed for colour, consistency, and presence of consolidation, haemorrhage, oedema, or parasitic nodules. The trachea and major bronchi were opened longitudinally and inspected for mucosal lesions and luminal contents. The liver was examined for size, colour, capsular integrity, and parenchymal texture, with representative sections made to assess the cut surface. The spleen was evaluated for size, colour, and consistency. The kidneys and adrenal glands were examined externally and sectioned transversally. The urinary bladder was opened and its content and mucosal surface were evaluated. In female specimens, the uterus and ovaries were examined; in males, the testes and accessory reproductive glands were assessed. The skeletal musculature was inspected for pallor, haemorrhage, or parasitic cysts, and representative sections of major muscle groups, including epaxial and hindlimb muscles. Finally, the skull was opened using bone scissors and a scalpel, and the brain was carefully extracted and examined for meningeal congestion, haemorrhage, or gross parenchymal lesions. The gastrointestinal tract, comprising the stomach and the entire length of the small and large intestine, was preserved intact and transferred to individually labelled containers for subsequent parasitological examination, including luminal content analysis and mucosal scraping for endoparasite detection.

Histological sampling was performed only on carcasses with a decomposition code ≤ 3.
Tissue samples (approximately 1 cm^3) were collected and fixed in 10% neutral buffered formalin, placed in dedicated containers, and stored in a ventilated cabinet until processing. Each container was labelled with SIMBAVET code (www.simbavet.org), squirrel identification code, date of necropsy and operator's initials.
The following tissues were collected in a single container: heart, liver (section), kidney, brain, intestine (section, opened and cleaned from content), urinary bladder, spleen, trachea (section).

For microbiological investigations, 6–7 sterile Eppendorf tubes (1.5–2.5 ml) were filled almost to capacity with tissue in order to ensure an adequate amount of material for analysis. Each tube was labelled with the SIMBAVET code, squirrel identification code, and organ. The tubes from each individual were placed in a sealed plastic bag labelled with the same identification codes and stored in a vertical freezer.

Figure 10. Post-mortem examination of a male Pallas's squirrel (*Callosciurus erythraeus*), specimen C319. Ventral view of the gross internal anatomy with visceral organs in situ following a midline incision.

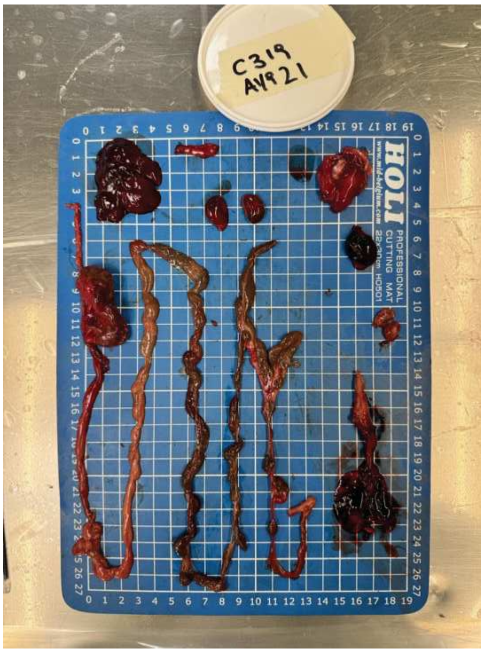


Figure 11. Macroscopic examination of harvested internal organs from specimen C319.

When carcasses were available unfrozen, bacteriological swabs were collected from the oral cavity, abdominal cavity and rectum. These procedures were performed only on the first freshly collected specimens (AY657, AY658, AY659). Bacterial strains were identified and evaluated using the MALDI-TOF scoring system. All recorded scores fell between 2.000 and 2.299, providing secure identification at the genus level and probable identification at the species level. To comprehensively screen the pathogens carried by the invasive population, a pooled sampling approach was employed.

CHAPTER 4
RESULTS

Our study found that Pallas's squirrels introduced to Italy carry a very low pathogen load, consistent with previous research on populations established outside their native range.
Of the 99 Pallas's squirrel samples collected, 54 were subjected to histological analysis.

4.1 Histopathological examination

The histopathological evaluation of the tissue samples from the Pallas's squirrels was affected by two main factors: severe post-mortem autolysis, which made many organs difficult to examine, and significant blood flow changes caused by the CO_2 euthanasia, as seen in in Table 5, Table 6 and Table 7. The findings are detailed below by organ system.

4.1.1 Lungs

The pulmonary parenchyma consistently exhibited moderate to severe diffuse edema (45/54), as seen in Figure 13, vascular congestion (4/54), and instances of blood extravasation within the alveolar spaces (1/54), as seen in Figure 12. These acute vascular changes are artifacts caused by the euthanasia procedure. They resulted from the hypoxia and increased capillary permeability induced by CO_2 inhalation. Independent of these terminal events, histopathological evaluation revealed pre-existing chronic respiratory alterations. Specifically, a mild multifocal lymphoplasmacytic bronchiolitis (1/54, specimen C302), as seen in Figure 14, and a moderate to severe pulmonary emphysema (1/54, specimen C375).

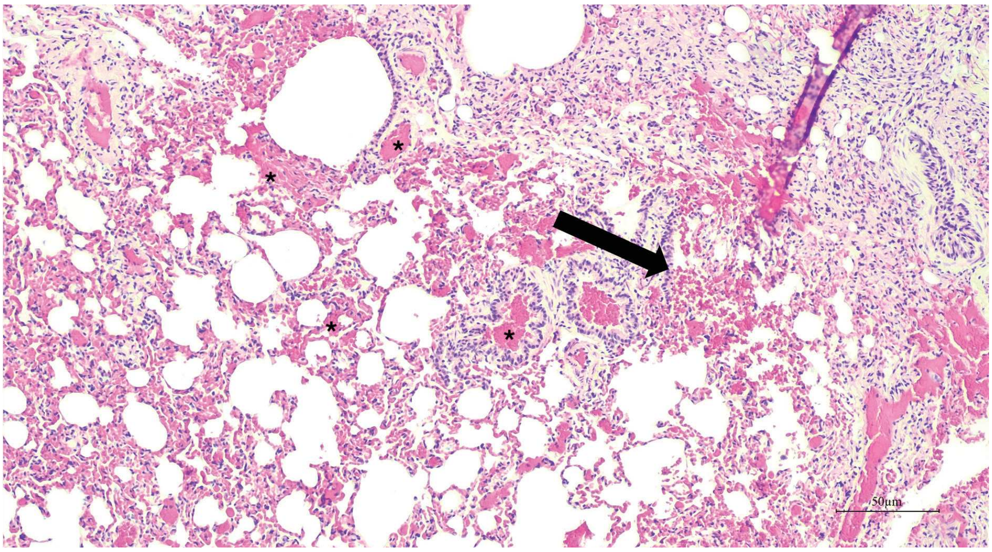


Figure 12. Lung, vascular congestion (asterisks) and haemorrhages in alveolar spaces (arrow). HE, 10X.

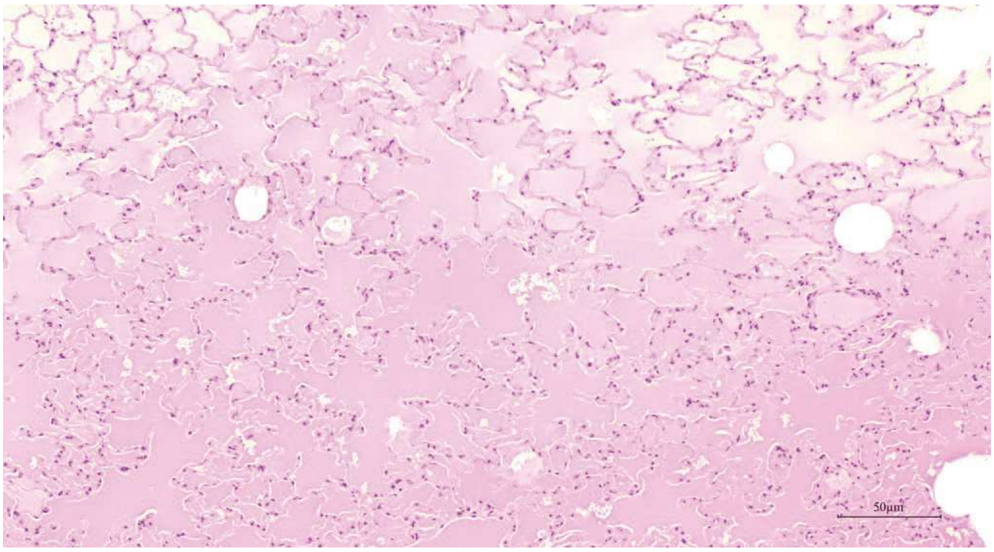


Figure 13. Lungs, dense eosinophilic material within the alveoli, severe pulmonary edema. HE, X10.

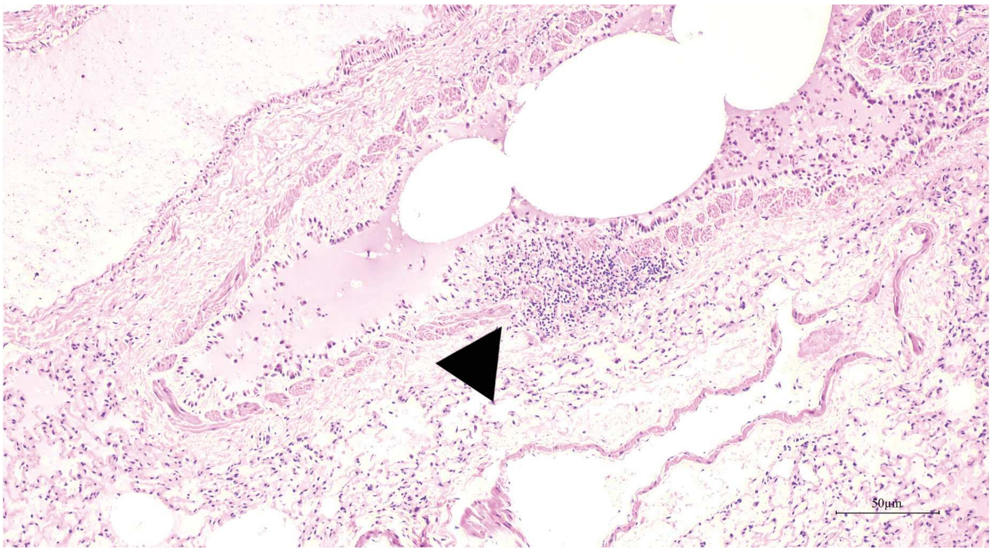
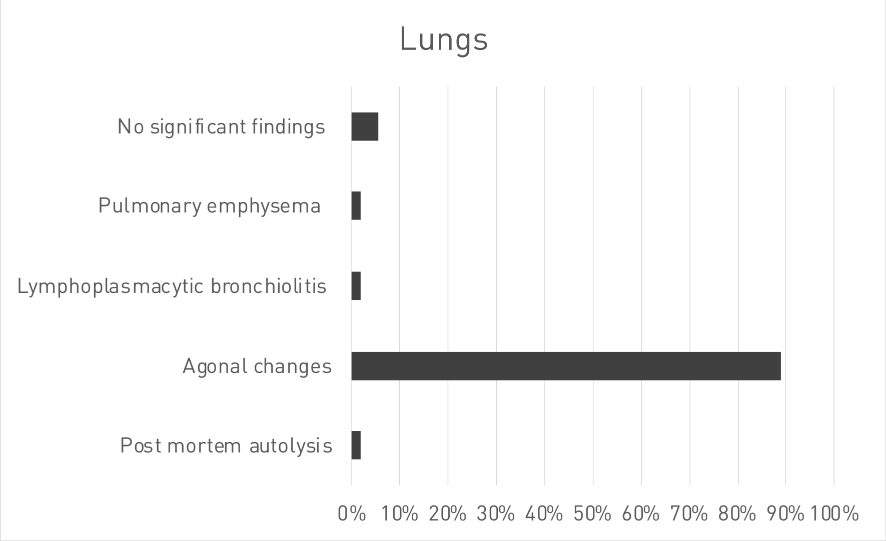


Figure 14. Lungs, peribronchial mild lymphocytic infiltrate (arrowhead). HE, X10.

Table 5. Prevalence of histopathological findings in the lungs of examined Pallas's squirrels (n = 54). The graph contrasts the high frequency of euthanasia-induced agonal changes (diffuse edema, vascular congestion, alveolar blood extravasation) with the minimal occurrence of pre-existing chronic respiratory alterations.



4.1.2 *Liver*

A widespread, moderate passive congestion was constantly observed, caused by blood backing up due to acute right heart failure as a consequence of the euthanasia process (13/54). Several specimens presented a severe diffused post-mortem autolysis (33/54). Severe vacuolar degeneration was observed in a liver section (1/54, specimen AY657) with multifocal areas of chronic granulomatous inflammation predominantly localized within the portal tracts. This infiltrate was of a mixed nature (neutrophilic and macrophagic) and was accompanied by cellular debris and the presence of multinucleated giant cells displaying a horseshoe nuclear conformation (Figure 15). Multifocal areas of neutrophilic and macrophagic inflammatory infiltrate associated with necrosis was detected in another specimen (1/54, specimen AY756), as seen in Figure 16.

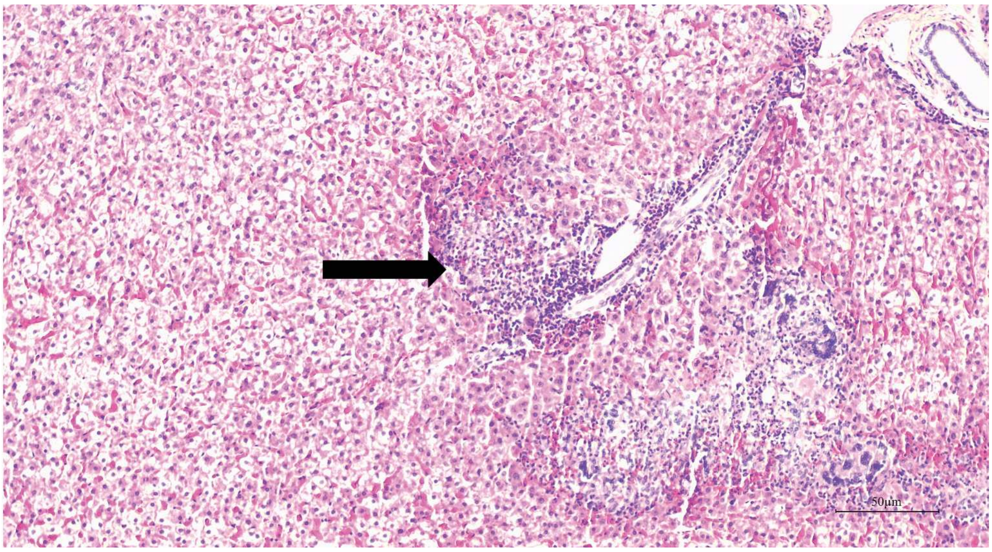


Figure 15. Liver, lymphoplasmacellular infiltrate (arrow), mild to moderate multifocal lymphoplasmacellular cholangio-hepatitis. HE, 10X.

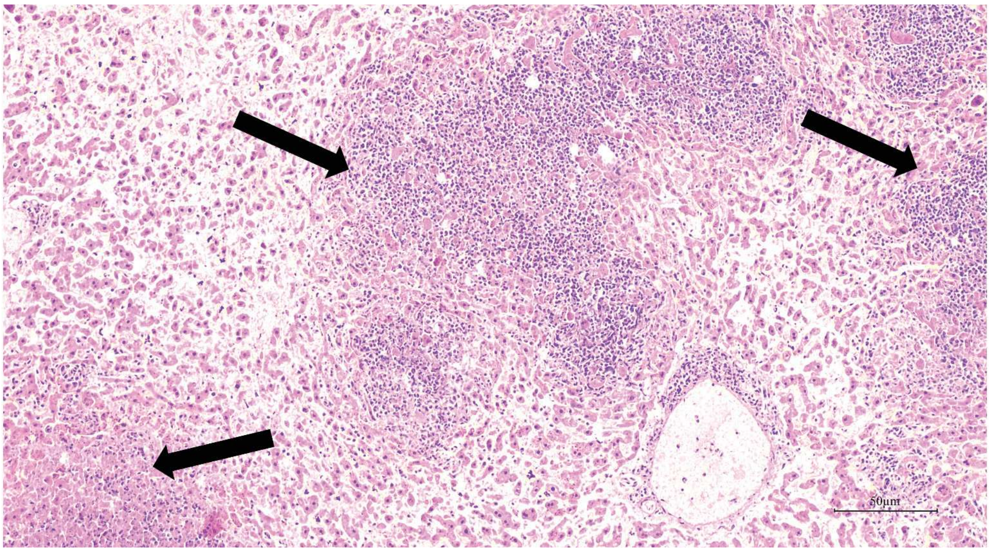
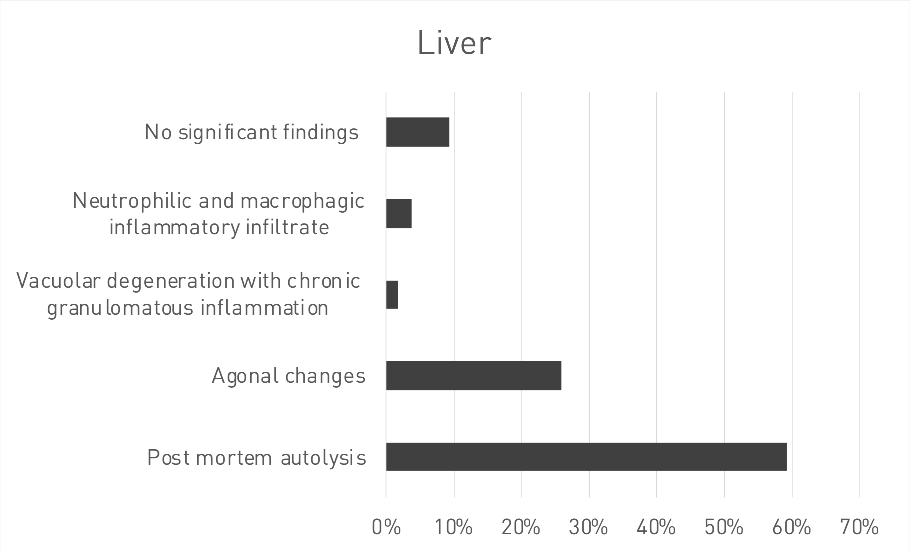


Figure 16. Liver, neutrophilic infiltrate (arrows), moderate to severe multifocal to coalescing necrotic hepatitis. HE, 10X.

Table 6. Prevalence of histopathological findings in the liver of examined Pallas's squirrels (n = 54). The chart details the percentage of specimens compromised by post-mortem autolytic processes and procedural artifacts compared to the low incidence of true pre-existing chronic lesions.



4.1.3 *Kidneys*

Examination of the renal tissue, in samples not entirely compromised by post-mortem autolytic processes allowed for the identification of pre-existing, chronic inflammatory and degenerative alterations (9/54, specimens AZ025, AY756, AZ059, AY658, AZ065, AZ069, AZ077, AZ519, AZ521). Specifically, the parenchyma exhibited focal to multifocal areas of interstitial nephritis (1/54, specimen AY756), as seen in Figure 17, and minimal to mild focal pyelonephritis (4/54, specimens AY658, AZ077, AZ519, AZ521). The inflammatory infiltrates were predominantly lymphoplasmacytic, indicating a chronic immune response. Additionally, multifocal micro-mineralizations were observed within the renal medulla (1/54, specimen AZ059), as seen in Figure 18.

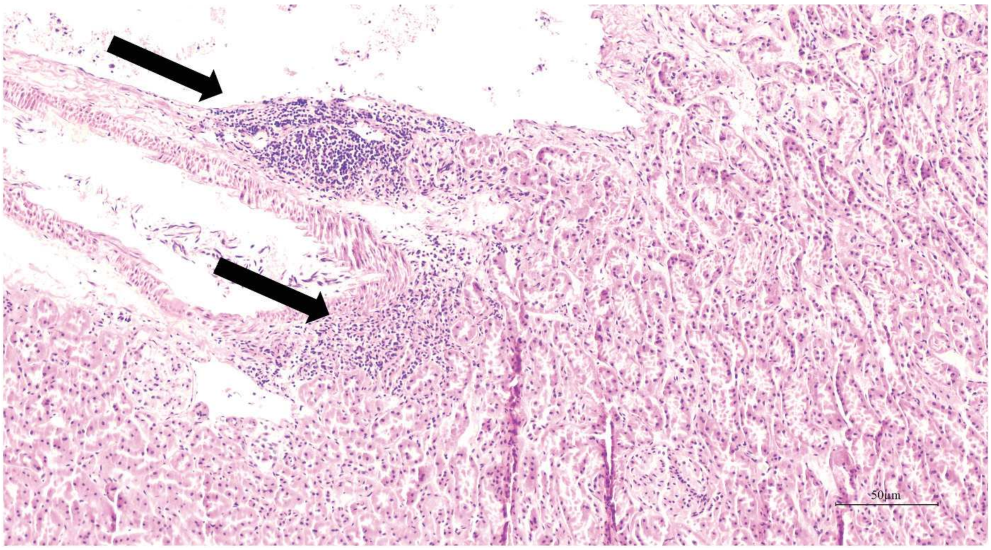


Figure 17. Kidney, lymphocytic infiltrate (arrow), mild multifocal interstitial nephritis. HE, 10X.

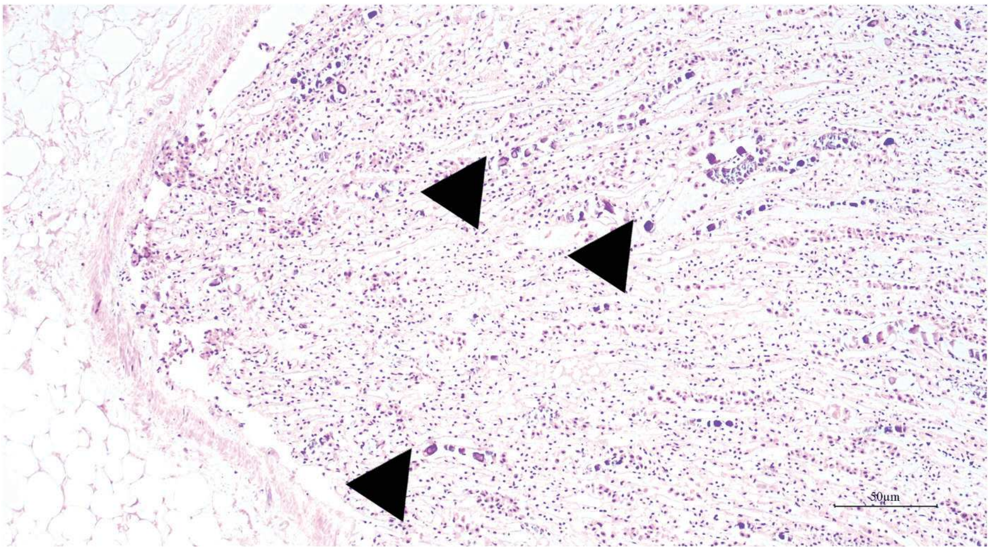
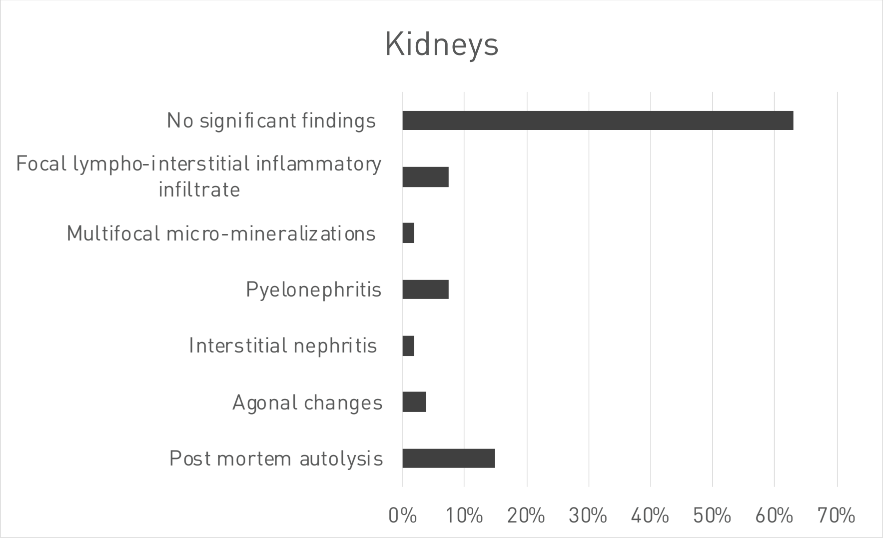


Figure 18. Kidney, multifocal mineralizations in the renal tubules (arrowheads). HE, 10X.

Table 7. Prevalence of histopathological findings in the kidneys of examined Pallas's squirrels (n = 54). The chart details the percentage of specimens compromised by post-mortem autolytic processes versus those presenting with pre-existing chronic inflammatory and degenerative alterations.



4.1.4 Heart

Examination of the heart muscle in some specimens was limited by post-mortem tissue degradation (7/54). The only finding in the was the accumulation of a brownish intracellular pigment within the cardiomyocytes (2/54), which is consistent with a formalin fixation artifact rather than a primary clinical pathology.

4.1.5 Central nervous system and gastrointestinal system

Histological analysis of the spleen, brain (CNS), and gastrointestinal tract (GIT) yielded no significant pathological findings. The evaluation of several specimens was limited by severe and diffuse post-mortem tissue degradation (Spleen: 4/54, GIT: 28/54, CNS: 7/54).

4.2 Bacteriological examination

Bacteriological analyses were performed on swabs collected exclusively from the three fresh squirrel carcasses (3/99: specimens AY657, AY658, and AY659), as seen in Table 8. Bacteriological findings from the oral cavity, rectum, and abdominal cavity of three Pallas's squirrel specimens examined.

i. Specimen AY657: examination of the oral cavity revealed a high-load of *Staphylococcus kloosii* (score 2.18) and the presence of *Streptococcus spp.*.

   In the anal cavity, *Escherichia coli*, *Enterococcus faecalis* (score 2.25), and *Staphylococcus spp.* were isolated. The abdominal cavity swab was negative, showing a complete absence of bacterial growth.

ii. Specimen AY658: for this subject, the oral swab showed a high load of *Streptococcus* spp. associated with a medium-load of *Haemophilus* spp..In the anal region, a high-load of both *Escherichia coli* and *Staphylococcus* spp. was detected. Unlike the other specimens, the swab taken from the abdominal cavity revealed a low-load growth of *Streptococcus* spp.

iii. Specimen AY659: analysis of the oral cavity confirmed a high-load growth of *Streptococcus spp.*. A high-load of *Streptococcus gallolyticus* (score 2.18) was isolated from the anal cavity, along with a medium-load of *Escherichia coli.* Similar to specimen AY657, the abdominal cavity swab showed no bacterial growth.

Table 8. Bacteriological findings from the oral cavity, rectum, and abdominal cavity of three Pallas's squirrel specimens examined. MALDI-TOF Score: Values between 2.000 and 2.299 indicate secure genus identification and probable species identification.

Species	Specimen	Sampling site	Result	Bacterial load	MALDI-TOF Score
Callosciurus erythraeus	AY657	Oral cavity	Staphylococcus kloosii	High	2.18
			Streptococcus spp.		
		Anal region	Escherichia coli		
			Enterococcus faecalis		2.25
			Staphylococcus spp.		
		Abdominal cavity	No growth		
Callosciurus erythraeus	AY658	Oral cavity	Streptococcus spp.	High	
			Haemophilus spp.	Medium	
		Anal region	Escherichia coli	High	
			Staphylococcus spp.	High	
		Abdominal cavity	Streptococcus spp.	Low	
Callosciurus erythraeus	AY659	Oral cavity	Streptococcus spp.	High	
		Anal region	Streptococcus gallolyticus	High	2.18
			Escherichia coli	Medium	
		Abdominal cavity	No growth		

4.3 Limitations of the study

The 99 examined squirrels were collected within a very narrow timeframe (January 10th to February 27th, 2025). Pathogen prevalence often shows seasonal fluctuations influenced by host physiology, climate-dependent vector life cycles (e.g. ticks and fleas), and the environmental persistence of microparasites. Therefore, sampling only during winter may have limited the detection of pathogens that typically peak in warmer months or during the reproductive period in spring and summer. This seasonal effect has already been demonstrated for some ectoparasites of squirrels: the flea *Ceratophyllus (s.) sciurorum* shows a significantly higher prevalence in autumn than in spring, while the tick *Ixodes ricinus* is significantly more prevalent in spring compared with the other seasons [13].

Histopathological evaluations were heavily compromised by severe post-mortem autolysis, which made tissues such as the heart muscle, spleen, brain, and gastrointestinal tract difficult to properly examine. The use of CO_2 for euthanasia induced acute vascular artifacts, resulting in significant blood flow changes such as diffuse edema, vascular congestion, and blood extravasation in the lungs, as well as passive congestion in the liver. In addition, bacteriological swabs were collected only from the first three freshly retrieved specimens, reducing the number of samples available for viable bacterial culture and likely leading to an underestimation of the bacterial diversity within the studied population.

Finally, the pooled sample technique, although efficient for large-scale screening and cost-effective for detecting low-prevalence pathogens, may produce a dilution effect. When a pathogen is present at a very low load in a single individual, mixing its tissues with those of uninfected animals can reduce the pathogen concentration below the detection limit of molecular or microbiological assays [54].

DISCUSSION

The exponential increase in biological invasions, driven by global trade and the movement of species, is fundamentally altering native ecosystems and facilitating the introduction of novel pathogens. In the last decade, the rate of alien species introductions has risen significantly, highlighting the urgent need for integrated ecological and epidemiological research [6], [3]. Within this context, the present study evaluated the epidemiological impact of the invasive Pallas's squirrel (*Callosciurus erythraeus*).

Necropsies of 99 carcasses revealed that Italian populations of *C. erythraeus* harbor a notably low parasite and pathogen load. Microbiological cultures predominantly yielded bacterial populations consistent with normal commensal flora, while histological evaluations highlighted only a few localized chronic inflammatory responses.

Several mechanisms may explain this reduced parasite richness in introduced populations of Pallas's squirrel. In Europe, these populations originate from releases or escapes from the pet trade and therefore likely derive from small founder groups, which reduces the probability of introducing their natural enemies. Moreover, animals involved in the pet trade are often treated with antiparasitic drugs, further decreasing the likelihood of co-introducing their original parasite fauna. Additionally, parasites that require high host densities or intermediate hosts may fail to establish in the new environment, while the arboreal habits of the species may limit its exposure to some parasites [13], [14], [39], [13], [55].

Overall, the invasion process may act as a filter that removes part of the original parasite community, a pattern consistent with the "enemy release hypothesis", according to which invasive species experience reduced parasitic pressure in the invaded range [14].

This conclusion is consistent with previous studies on introduced populations outside their native range, which have reported low pathogen prevalence in Pallas's squirrels.

In Italy, ectoparasites represent the most frequently detected infectious agents in Pallas's squirrels. The most common species identified were the flea *Ceratophyllus sciurorum sciurorum* and the generalist tick *Ixodes ricinus.* These ectoparasites could represent a potential pathway for pathogen transmission between invasive and native squirrels. In particular, I. ricinus is an important vector of several pathogens of veterinary and public health relevance, meaning that Pallas's squirrels could potentially influence the transmission dynamics of tick-borne diseases [13].

Gastrointestinal helminths and microparasites show very low prevalence and high host specificity [13], [46], [51]. Regarding viruses, the Italian population tested negative for the zoonotic Variegated Squirrel Bornavirus 1 (VSBV-1). However, three novel viruses were identified: one polyomavirus and two herpesviruses, probably introduced to Italy through the exotic pet trade [53].

Most of the pathogens recorded in epidemiological studies appear to have been acquired after the establishment of the species in the invaded environment rather than being co-introduced from the native range. The only likely exception is the nematode *Strongyloides callosciureus,* a parasite specific to sciurids that appears to have been introduced together with Pallas's squirrels [13]. However, its prevalence is extremely low (1%), and it has not yet been reported in native squirrel species. Although some studies suggest that this parasite may have a relatively broad host potential [56], confirmed records are still largely restricted to sciurids [13].

In conclusion, evidence of parasite exchange between native and invasive squirrels appears to be limited. Some cases of spillover from the native red squirrel to the invasive Pallas's squirrel have been documented, such as Trypanoxyuris sciuri and the flea *Ceratophyllus sciurorum sciurorum*. However, spillback from Pallas's squirrels to native species has not yet been observed [13], suggesting that active interspecific transmission is currently minimal and that the immediate sanitary risk remains low.

However, these findings should be interpreted as part of a dynamic rather than a stable situation. Host–parasite relationships require time to develop, and evidence shows that invasive species tend to accumulate parasites as their populations expand and persist in the invaded area. Repeated introduction events can further increase the probability of co-introducing pathogens from the native range [37], [55]. In this context, the ban on the trade and release of this species in Italy represents a fundamental measure to prevent new introductions and reduce future sanitary risks.

It is important to consider that an invasive species may remain apparently harmless for a long time and become problematic only after environmental changes. For example, warmer temperatures in northern Europe have allowed the development of Dirofilaria nematodes, leading to a sudden increase in infections in dogs and humans. Therefore, a prolonged phase of low impact should not be interpreted as evidence of a low future risk. Following the precautionary principle, as stated in the Convention on Biological Diversity, very invasive species should be considered a potential source of undesirable effects, even when there is no immediate evidence [36]. In addition, invasive species do not necessarily need to introduce new pathogens to have a sanitary impact. They can also modify habitats and ecological relationships in ways that facilitate the spread of local diseases. These mechanisms are often difficult to detect in the early stages.

It should also be noted that although *C. erythraeus* has a low pathogen load, this does not mean that it is harmless. Release from natural enemies may enhance its competitive advantage over the native red squirrel. Unlike the native species, which must invest energy in coping with a complex parasite community, the invasive squirrel may have more energy available for survival, reproduction, and space occupation. This advantage may promote population growth and increase competition with *S. vulgaris*, contributing to its decline in invaded areas [18], [31].

The discussion about invasive alien species has gained increasing attention in the last decade, but preventive measures are still far from effective in reducing their introduction. The rising trend of new introductions highlights the need for stronger management efforts focused on prevention, which represents the most cost-effective strategy, together with increased research, monitoring, and public education.

The management of invasive alien species is not only a scientific and institutional issue but also a social one. Public awareness plays a crucial role in preventing new introductions and in supporting control and eradication programs. Informing citizens about the risks associated with invasive species and promoting responsible behaviors, particularly in relation to the pet trade and the release of animals, is essential for the long-term effectiveness of management strategies. One of the main challenges in addressing biological invasions lies in public perception. Public awareness of invasive alien species is often limited, and many people underestimate the risks they pose. Even among those who recognize these threats, support for control measures is often low, especially when the species involved is visually attractive. Perception is also important among professionals: for a species to be formally recognized as invasive and prioritized for management, it must be widely perceived as problematic. Species that primarily affect specific sectors rather than public health are often treated as niche issues rather than broader management priorities [5], [6].

Initiatives aimed at increasing public awareness about the health risks associated with invasive alien species are therefore essential. Such efforts can promote responsible behavior when crossing borders and improve public support for IAS control and eradication programs.

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APPENDIX A

ID	SIMBAVET	ENTRY DATE	AGE	SPECIES	SEX	WEIGHT	OWNER	ORIGIN	PROVINCE	NECROPSY DATE	HISTOLOGY	STORAGE	SAMPLING METHOD	BACTERIOLOGY
C373	AY657	27/02/2025	Adult	Scoiattolo di pallas	M	260	UNINSUBRIA	Rancio Valcuvia	VA	27/02/2025	SI	amb	singoli organi	SI
C374	AY658	27/02/2025	Adult	Scoiattolo di pallas	F	280	UNINSUBRIA	Rancio Valcuvia	VA	27/02/2025	SI	amb	singoli organi	SI
C375	AY659	27/02/2025	Adult	Scoiattolo di pallas	F	270	UNINSUBRIA	Villaggio olandese	VA	27/02/2025	SI	amb	singoli organi	SI
C349	AY751	12/03/2025	Adult	Scoiattolo di pallas	F	250	UNINSUBRIA	Cassano Valcuvia	VA	12/03/2025	SI	-20	singoli organi	
C354	AY753	12/03/2025	Adult	Scoiattolo di pallas	F	280	UNINSUBRIA	Montegrino	VA	12/03/2025	SI	-20	singoli organi	
C355	AY754	12/03/2025	Adult	Scoiattolo di pallas	F	275	UNINSUBRIA	Bosco Roggiano	VA	12/03/2025	SI	-20	singoli organi	
C315	AY755	12/03/2025	Adult	Scoiattolo di pallas	F	290	UNINSUBRIA	Roggiano	VA	12/03/2025	SI	-20	singoli organi	
C317	AY756	12/03/2025	Adult	Scoiattolo di pallas	M	305	UNINSUBRIA	Roggiano	VA	12/03/2025	SI	-20	singoli organi	
C346	AY757	12/03/2025	Adult	Scoiattolo di pallas	M	305	UNINSUBRIA	Az. Ratti	VA	12/03/2025	SI	-20	singoli organi	
C348	AY758	12/03/2025	Adult	Scoiattolo di pallas	F	275	UNINSUBRIA	Cassano Valcuvia	VA	12/03/2025	SI	-20	singoli organi	
C358	AY759	12/03/2025	Adult	Scoiattolo di pallas	M	270	UNINSUBRIA	Montegrino	VA	12/03/2025	SI	-20	singoli organi	
C343	AY760	12/03/2025	Adult	Scoiattolo di pallas	F	280	UNINSUBRIA	Cassano Valcuvia	VA	12/03/2025	SI	-20	singoli organi	
C289	AY761	12/03/2025	Adult	Scoiattolo di pallas	F	255	UNINSUBRIA	Roggiano	VA	12/03/2025	SI	-20	singoli organi	
C296	AY762	12/03/2025	Adult	Scoiattolo di pallas	M	270	UNINSUBRIA	Bosco delle Fate	VA	12/03/2025	SI	-20	singoli organi	
/	AY764	12/03/2025	Adult	Scoiattolo di pallas	M	280	UNINSUBRIA	/	VA	12/03/2025	SI	-20	singoli organi	
C316	AY765	12/03/2025	Adult	Scoiattolo di pallas	F	265	UNINSUBRIA	Roggiano	VA	12/03/2025	SI	-20	singoli organi	
C319	AY921	27/03/2025	Adult	Scoiattolo di pallas	M	280	UNINSUBRIA	Roggiano	VA	27/03/2025		-20	singoli organi	
C292	AY916	28/03/2025	Adult	Scoiattolo di pallas	F	270	UNINSUBRIA	Roggiano	VA	28/03/2025		-20	singoli organi	
C330	AY922	29/03/2025	Adult	Scoiattolo di pallas	M	285	UNINSUBRIA	Privato casa bianca	VA	29/03/2025		-20	singoli organi	
C335	AY924	30/03/2025	Adult	Scoiattolo di pallas	M	305	UNINSUBRIA	Ditta Ratti	VA	30/03/2025		-20	singoli organi	
C334	AY923	31/03/2025	Adult	Scoiattolo di pallas	F	260	UNINSUBRIA	Ditta Ratti	VA	31/03/2025		-20	singoli organi	
C303	AY920	01/04/2025	Adult	Scoiattolo di pallas	F	280	UNINSUBRIA	Privato Germignaga	VA	01/04/2025		-20	singoli organi	
C352	AY925	02/04/2025	Adult	Scoiattolo di pallas	F	275	UNINSUBRIA	Ditta Ratti	VA	02/04/2025		-20	singoli organi	
C308	AY918	03/04/2025	Adult	Scoiattolo di pallas	M	295	UNINSUBRIA	Cassano Valcuvia-sotsass	VA	03/04/2025		-20	singoli organi	
C309	AY919	04/04/2025	Adult	Scoiattolo di pallas	M	320	UNINSUBRIA	Cassano Valcuvia-sotsass	VA	04/04/2025		-20	singoli organi	
C294	AY917	05/04/2025	Adult	Scoiattolo di pallas	F	255	UNINSUBRIA	Bosco Fate	VA	05/04/2025		-20	singoli organi	
C286	AZ022	09/04/2025	Adult	Scoiattolo di pallas	F	220	UNINSUBRIA	Bosco Fate	VA	09/04/2025	SI	-20	singoli organi	
C291	AZ023	09/04/2025	Adult	Scoiattolo di pallas	F	290	UNINSUBRIA	Bosco Fate	VA	09/04/2025	SI	-20	singoli organi	
C293	AZ024	09/04/2025	Adult	Scoiattolo di pallas	F	270	UNINSUBRIA	Privato Roggiano	VA	09/04/2025	SI	-20	singoli organi	
C298	AZ025	09/04/2025	Adult	Scoiattolo di pallas	M	290	UNINSUBRIA	Bosco Cantevria	VA	09/04/2025	SI	-20	singoli organi	
C299	AZ026	09/04/2025	Adult	Scoiattolo di pallas	F	265	UNINSUBRIA	Privato Germignaga	VA	09/04/2025	SI	-20	singoli organi	
C300	AZ027	09/04/2025	Adult	Scoiattolo di pallas	M	275	UNINSUBRIA	Privato Germignaga	VA	09/04/2025	SI	-20	singoli organi	
C301	AZ028	09/04/2025	Adult	Scoiattolo di pallas	M	285	UNINSUBRIA	Privato Germignaga	VA	09/04/2025	SI	-20	singoli organi	
c302	AZ029	09/04/2025	Adult	Scoiattolo di pallas	F	200	UNINSUBRIA	Privato roggiano	VA	09/04/2025	SI	-20	singoli organi	
C305	AZ030	09/04/2025	Adult	Scoiattolo di pallas	M	290	UNINSUBRIA	Privato Roggiano	VA	09/04/2025	SI	-20	singoli organi	
C307	AZ031	09/04/2025	Adult	Scoiattolo di pallas	M	285	UNINSUBRIA	Privato Germignaga	VA	09/04/2025	SI	-20	singoli organi	
C310	AZ032	09/04/2025	Adult	Scoiattolo di pallas	F	250	UNINSUBRIA	Bosco Cantevria	VA	09/04/2025		-20	singoli organi	
C311	AZ033	09/04/2025	Adult	Scoiattolo di pallas	F	195	UNINSUBRIA	Privato casa bianca	VA	09/04/2025	SI	-20	singoli organi	
C312	AZ034	09/04/2025	Adult	Scoiattolo di pallas	F	275	UNINSUBRIA	Privato Germignaga	VA	09/04/2025		-20	singoli organi	
C313	AZ035	09/04/2025	Adult	Scoiattolo di pallas	M	295	UNINSUBRIA	Privato Germignaga	VA	09/04/2025		-20	singoli organi	
C314	AZ036	09/04/2025	Adult	Scoiattolo di pallas	M	290	UNINSUBRIA	Privato casa bianca	VA	09/04/2025	SI	-20	singoli organi	
C318	AZ037	09/04/2025	Adult	Scoiattolo di pallas	M	260	UNINSUBRIA	Privato casa bianca	VA	09/04/2025	SI	-20	singoli organi	
C320	AZ038	09/04/2025	Adult	Scoiattolo di pallas	F	285	UNINSUBRIA	Cassano Valcuvia-sotsass	VA	09/04/2025	SI	-20	singoli organi	
C321	AZ039	09/04/2025	Adult	Scoiattolo di pallas	M	280	UNINSUBRIA	Scuola Roggiano	VA	09/04/2025	SI	-20	singoli organi	
C322	AZ040	09/04/2025	Adult	Scoiattolo di pallas	F	260	UNINSUBRIA	Bosco Fate	VA	09/04/2025		-20	singoli organi	
C323	AZ041	09/04/2025	Adult	Scoiattolo di pallas	M	285	UNINSUBRIA	Bosco Fate	VA	09/04/2025	SI	-20	singoli organi	
C324	AZ042	09/04/2025	Adult	Scoiattolo di pallas	M	290	UNINSUBRIA	Privato Germignaga	VA	09/04/2025	SI	-20	singoli organi	
C325	AZ043	09/04/2025	Adult	Scoiattolo di pallas	F	305	UNINSUBRIA	Ditta Ratti	VA	09/04/2025	SI	-20	singoli organi	
C326	AZ044	09/04/2025	Adult	Scoiattolo di pallas	M	285	UNINSUBRIA	Ditta Ratti	VA	09/04/2025		-20	singoli organi	
C327	AZ045	09/04/2025	Adult	Scoiattolo di pallas	M	285	UNINSUBRIA	Bosco Cantevria	VA	09/04/2025		-20	singoli organi	
C328	AZ046	09/04/2025	Adult	Scoiattolo di pallas	M	310	UNINSUBRIA	Scuola Roggiano	VA	09/04/2025		-20	singoli organi	
C329	AZ047	09/04/2025	Adult	Scoiattolo di pallas	M	285	UNINSUBRIA	Scuola Roggiano	VA	09/04/2025		-20	singoli organi	
C331	AZ048	09/04/2025	Adult	Scoiattolo di pallas	F	265	UNINSUBRIA	Scuola Roggiano	VA	09/04/2025		-20	singoli organi	
C332	AZ049	09/04/2025	Adult	Scoiattolo di pallas	M	290	UNINSUBRIA	Bosco Cantevria	VA	09/04/2025		-20	singoli organi	
C333	AZ050	09/04/2025	Adult	Scoiattolo di pallas	M	305	UNINSUBRIA	Privato Germignaga	VA	09/04/2025		-20	singoli organi	
C336	AZ051	09/04/2025	Adult	Scoiattolo di pallas	M	285	UNINSUBRIA	Bosco Cantevria	VA	09/04/2025		-20	singoli organi	
C338	AZ052	09/04/2025	Adult	Scoiattolo di pallas	F	260	UNINSUBRIA	Privato Roggiano	VA	09/04/2025		-20	singoli organi	
C340	AZ053	09/04/2025	Adult	Scoiattolo di pallas	M	330	UNINSUBRIA	Cassano Valcuvia-sotsass	VA	09/04/2025	SI	-20	singoli organi	
C341	AZ054	09/04/2025	Adult	Scoiattolo di pallas	F	235	UNINSUBRIA	Privato Roggiano	VA	09/04/2025		-20	singoli organi	
C342	AZ055	09/04/2025	Adult	Scoiattolo di pallas	M	285	UNINSUBRIA	Scuola Roggiano	VA	09/04/2025		-20	singoli organi	
C359	AZ056	09/04/2025	Adult	Scoiattolo di pallas	M	265	UNINSUBRIA	Montegrino	VA	09/04/2025		-20	singoli organi	
C363	AZ057	09/04/2025	Adult	Scoiattolo di pallas	M	270	UNINSUBRIA	Villaggio Olandese	VA	09/04/2025		-20	singoli organi	
C365	AZ058	09/04/2025	Adult	Scoiattolo di pallas	M	230	UNINSUBRIA	Rancio Valcuvia	VA	09/04/2025		-20	singoli organi	
C368	AZ059	09/04/2025	Adult	Scoiattolo di pallas	M	315	UNINSUBRIA	Ditta Ratti	VA	09/04/2025	SI	-20	singoli organi	
C371	AZ060	09/04/2025	Adult	Scoiattolo di pallas	F	270	UNINSUBRIA	Ditta Ratti	VA	09/04/2025	SI	-20	singoli organi	
C372	AZ061	09/04/2025	Adult	Scoiattolo di pallas	M	240	UNINSUBRIA	Stalletti tetti	VA	09/04/2025		-20	singoli organi	
C282	AZ062	09/04/2025	Adult	Scoiattolo di pallas	/	/	UNINSUBRIA	/	VA	09/04/2025	SI	-20	singoli organi	
C284	AZ063	09/04/2025	Adult	Scoiattolo di pallas	/	/	UNINSUBRIA	/	VA	09/04/2025		-20	singoli organi	
SP4	AZ065	09/04/2025	Adult	Scoiattolo di pallas	/	/	UNINSUBRIA	/	VA	09/04/2025	SI	-20	singoli organi	
SP5	AZ066	09/04/2025	Adult	Scoiattolo di pallas	/	/	UNINSUBRIA	/	VA	09/04/2025		-20	singoli organi	
SP6	AZ067	09/04/2025	Adult	Scoiattolo di pallas	/	/	UNINSUBRIA	/	VA	09/04/2025		-20	singoli organi	
SP7	AZ068	09/04/2025	Adult	Scoiattolo di pallas	/	/	UNINSUBRIA	/	VA	09/04/2025		-20	singoli organi	
SP8	AZ069	09/04/2025	Adult	Scoiattolo di pallas	/	/	UNINSUBRIA	/	VA	09/04/2025	SI	-20	singoli organi	
SP9	AZ070	09/04/2025	Adult	Scoiattolo di pallas	/	/	UNINSUBRIA	/	VA	09/04/2025		-20	singoli organi	
SP10	AZ071	09/04/2025	Adult	Scoiattolo di pallas	/	/	UNINSUBRIA	/	VA	09/04/2025		-20	singoli organi	
SP11	AZ072	09/04/2025	Adult	Scoiattolo di pallas	/	/	UNINSUBRIA	/	VA	09/04/2025		-20	singoli organi	
SP12	AZ073	09/04/2025	Adult	Scoiattolo di pallas	/	/	UNINSUBRIA	/	VA	09/04/2025		-20	singoli organi	
SP13	AZ074	09/04/2025	Adult	Scoiattolo di pallas	/	/	UNINSUBRIA	/	VA	09/04/2025		-20	singoli organi	
SP14	AZ075	09/04/2025	Adult	Scoiattolo di pallas	/	/	UNINSUBRIA	/	VA	09/04/2025	SI	-20	singoli organi	
SP15	AZ076	09/04/2025	Adult	Scoiattolo di pallas	/	/	UNINSUBRIA	/	VA	09/04/2025		-20	singoli organi	
SP16	AZ077	09/04/2025	Adult	Scoiattolo di pallas	/	/	UNINSUBRIA	/	VA	09/04/2025	SI	-20	singoli organi	
SP17	AZ078	09/04/2025	Adult	Scoiattolo di pallas	/	/	UNINSUBRIA	/	VA	09/04/2025		-20	singoli organi	
SP18	AZ404	20/05/2025	Adult	Scoiattolo di pallas	/	/	UNINSUBRIA	/	VA	20/05/2025	SI	-20	singoli organi	
SP19	AZ405	20/05/2025	Adult	Scoiattolo di pallas	/	/	UNINSUBRIA	/	VA	20/05/2025		-20	singoli organi	
SP20	AZ406	20/05/2025	Adult	Scoiattolo di pallas	/	/	UNINSUBRIA	/	VA	20/05/2025	SI	-20	singoli organi	
SP21	AZ407	20/05/2025	Adult	Scoiattolo di pallas	/	/	UNINSUBRIA	/	VA	20/05/2025	SI	-20	singoli organi	
SP22	AZ408	20/05/2025	Adult	Scoiattolo di pallas	/	/	UNINSUBRIA	/	VA	20/05/2025		-20	singoli organi	
SP23	AZ409	20/05/2025	Adult	Scoiattolo di pallas	/	/	UNINSUBRIA	/	VA	20/05/2025	SI	-20	singoli organi	
SP24	AZ411	20/05/2025	Adult	Scoiattolo di pallas	/	/	UNINSUBRIA	/	VA	20/05/2025	SI	-20	singoli organi	
SP25	AZ410	20/05/2025	Adult	Scoiattolo di pallas	/	/	UNINSUBRIA	/	VA	20/05/2025		-20	singoli organi	
SP26	AZ412	20/05/2025	Adult	Scoiattolo di pallas	/	/	UNINSUBRIA	/	VA	20/05/2025	SI	-20	singoli organi	
C439	AZ519	29/05/2025	Adult	Scoiattolo di pallas	M	248	UNINSUBRIA	/	VA	29/05/2025	SI	-20	singoli organi	
C460	AZ520	29/05/2025	Adult	Scoiattolo di pallas	F	228	UNINSUBRIA	/	VA	29/05/2025	SI	-20	singoli organi	
C471	AZ521	29/05/2025	Adult	Scoiattolo di pallas	F	316	UNINSUBRIA	/	VA	29/05/2025	SI	-20	singoli organi	
C433	AZ522	29/05/2025	Adult	Scoiattolo di pallas	M	313	UNINSUBRIA	/	VA	29/05/2025	SI	-20	singoli organi	
C448	AZ523	29/05/2025	Adult	Scoiattolo di pallas	F	299	UNINSUBRIA	/	VA	29/05/2025	SI	-20	singoli organi	
C466	AZ524	29/05/2025	Adult	Scoiattolo di pallas	F	281	UNINSUBRIA	/	VA	29/05/2025	SI	-20	singoli organi	
C461	AZ525	29/05/2025	Adult	Scoiattolo di pallas	F	277	UNINSUBRIA	/	VA	29/05/2025				
C465	AZ526	29/05/2025	Adult	Scoiattolo di pallas	F	295	UNINSUBRIA	/	VA	29/05/2025				