UNIVERSITÀ DEGLI STUDI DI MILANO

CORSO DI DOTTORATO IN EPIDEMIOLOGIA AMBIENTE E SANITA PUBBLICA
XXXIII ciclo

DIPARTIMENTO DI SCIENZE CLINICHE E DI COMUNITA'

TESI DI DOTTORATO DI RICERCA

Disease risk assessment of Invasive Alien Species

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A.A.
2019-2020

It is better to be healthy than ill or dead.
That is the beginning and the end of the only real argument for preventive medicine.
It is sufficient.

Geoffrey Rose, 1992

Abstract

Increased global trade and travel have led to a rise in the number of invasive alien species (IAS), i.e. species introduced by humans in geographic areas where are not naturally found, worldwide. Despite the recognized role of wildlife, as well as of wildlife translocations, in the emergence and re-emergence of infections of public health significance, IAS remain mainly studied for their environmental impacts, and their disease risk towards humans and animals is still largely neglected by health professionals.

The main aim of this thesis is therefore to cover this gap by setting the ground for a new "invasion epidemiology" field, and this has been done through two main steps: the review and analysis of both the mechanisms underlying IAS disease risk and the information available in literature on IAS pathogens, and the development of a standardized qualitative disease risk assessment method, applicable to different geographic contexts, to assess the risk of mammal IAS to impact on human and animal health.

First, I reviewed the existing biological and ecological literature on IAS to identify the main mechanisms by which animal IAS may affect disease risk in their area of release. IAS resulted to potentially affect disease risk both directly, by acting as hosts of infectious agents, thus possibly leading to the introduction of new pathogens, and/or the amplification of endemic ones, or indirectly, by altering the ecosystem equilibrium, through competitive and trophic interactions with native host species or the modification of local habitats. This literature review highlighted how IAS may have important health implications, which should be better acknowledged by people working in the human and animal health field, and how the mechanisms underlying the sanitary outcome of a biological invasion, and in particular indirect ones, are extremely complex, being the product of multiple factors. Acknowledging the important limitations of our current ability to predict possible health impacts driven by indirect mechanisms, I decided to address the issue of IAS disease risk by focusing specifically on IAS possible role as infectious agents' host.

As information on IAS pathogens is not systematized, preventing from knowing the amount and quality of available data to inform possible disease risk assessment procedures, I systematically reviewed the literature on the infectious agents of the main mammal species of European Union concern. Current knowledge on the pathogens harbored by mammal IAS was evaluated through different statistical approaches: the identification of the main factors associated with research intensity and the observed pathogen species richness, the estimation of the true pathogen species richness, and a meta-analysis of prevalence of the pathogens of public and animal health significance. Results highlighted the existence of strong information gaps and biases in the way research on mammal IAS pathogens is carried out, the current underestimation of the amount of pathogens harbored by these species and high levels of uncertainty in the pooled prevalence of pathogens of public and animal health significance. However, the review confirmed that mammal IAS harbor pathogens of human and animal health significance, and therefore, the need to identify high-risk species.

Considering that the existing knowledge gaps would have resulted in strong limitations in informing a risk assessment procedure, I developed a qualitative disease risk assessment methodology informed by expert opinion. This tool is specifically aimed at assessing IAS disease risk towards humans, domestic animal populations, and/or wildlife populations and allows to obtain a list of the pathogens of animal and human health significance that mammal IAS could transmit to a population of interest (directly or through the communities of local hosts), each with the related level of risk and uncertainty. Key features of the tool are its flexibility, being applicable to different contexts and for different purposes, and the high resolution of the mechanisms under assessment, which make possible for risk managers identifying the most critical pathogens and mechanisms involved in disease risk, allowing them to direct targeted actions and surveillance plans.

Finally, the need to combine multiple likelihood estimates deriving from several pathways in an overall risk estimate led me to tackle a methodological aspect of qualitative risk assessment procedures, and I proposed a standardized method applicable in such cases, to reduce the subjectivity that relies in the different ways multiple estimates are currently combined.

Overall, this thesis highlights how our knowledge of the role of IAS in disease dynamics might be currently underestimated, and the urgent necessity to identify the species at highest priority to direct empirical research and preventive actions, despite the scarcity of data. In a changing and connected world, prevention of possible future health threats should be treated as a guiding principle, and not as an option.

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CHAPTER 1

General introduction

1. New threats in a globalized world: Invasive Alien Species

Biological invasions represent one of the drawbacks of the increased global connection. Alien organisms (animals or plants), introduced outside their native range by humans, can succeed in adapting and establishing in the new environment, and possibly spread uncontrollably with harmful consequences for local ecosystems, thus becoming "invasive" (Kolar and Lodge, 2002).

Since the 1800s, in parallel with the increase of travel and trade activities, the number of introduction events of species in new areas has risen dramatically, and this trend is not expected to change (Seebens *et al.*, 2017). In Europe only, the number of alien species has increased of the 76% in the 1970-2007 period (Genovesi *et al.*, 2009), with currently more than 14.000 alien species present, of which the 10-15% invasive (European Union, 2014a).

Invasive alien species (IAS) are a major environmental, economic and social concern (European Union, 2014a). First, they are one of the main drivers of biodiversity loss worldwide after habitat loss and fragmentation (Vitousek *et al.*, 1997). A recent analysis of the drivers of extinctions in five major taxa (plants, amphibians, reptiles, birds and mammals) showed that alien species are the second most common threat associated with species extinction (Bellard, Cassey and Blackburn, 2016). IAS can indeed harm native species populations through several mechanisms, including predation, competition for limited resources, inter-breeding, and the transmission of diseases. Moreover, IAS may compromise the ability of local ecosystems to provide their benefits to human well-being (the so-called "ecosystem services"), such as pollination, water regulation or flood control (European Union, 2014a).

Biological invasions cause also significant impacts on a number of economic activities, like agriculture, forestry and fisheries, through the damage of infrastructure and the destruction of landscapes and water bodies. Estimates suggest that monetary impacts of IAS in Australia, Brazil, India, South Africa, the United Kingdom and the United States are in the range of 300 billion dollars per year (Pimentel *et al.*, 2001; Pimentel, Zuniga and Morrison, 2005). In Europe, economic costs related to both damages caused by IAS and control actions, have been estimated in about 12.5 billion euros per year (Kettunen *et al.*, 2009).

Lastly, IAS can represent a major problem for human health, acting as vectors for dangerous pathogens and triggering allergies and skin problems (European Union, 2014a).

Unfortunately, eradication is extremely difficult once IAS have established, often making their impacts on local species and habitats irreversible. For this reason, prevention has been recognized as the best strategy to cope with biological invasions, and it is at the base of the current guidelines and strategies to tackle IAS developed by international organizations and national and international governments.

The European Union has recently issued Regulation N° 1143/2014 "on the prevention and management of the introduction and spread of invasive alien species", which is the first comprehensive legal framework dealing with IAS at Union level. The regulation is structured around three main pillars: (i) prevention, through the analysis of the pathways of unintentional introduction and spread of IAS, (ii) early warning and rapid response to newly establishing IAS, thanks to an official surveillance system, and (iii) management of already established IAS through a series of measures to control, contain or eradicate them (European Union, 2014b). These

measures focus on a specific list of invasive species of Union concern, defined with the Commission Implementing Regulations (EU) 2016/1141 (European Union, 2016), (EU) 2017/1263 (European Union, 2017) and (EU) 2019/1262 (European Union, 2019), which is regularly updated in collaboration with the Member States. The list has to comprehend all the species with the most significant social, economic and biodiversity impacts, as defined through appropriate risk assessments carried out based on common criteria, and it is currently comprehensive of 66 species (30 animal species and 36 plant species).

1.1 Animal IAS and health

IAS have the potential to affect health in several ways. Mazza *et al.* (2014) identified four main categories of modalities by which IAS may threaten human health:

- Causing diseases or infections;
- Exposing humans to wounds from bites/stings, biotoxins, allergens or toxicants;
- Faciliting diseases, injuries or death;
- Causing other negative effects on human livelihood.

For example, *Vespa velutina nigrithorax*, a hornet introduced from Asia to France, from where it spread to other European countries including Italy, may cause serious allergic reactions and anaphylaxis (Chugo *et al.*, 2015). The American crayfish *Procrambarus clarkii* is known to bioaccumulate toxins and heavy metals, to which humans may be exposed through food (Gherardi *et al.*, 2002; Tricarico *et al.*, 2008).

Some IAS may even cause human death: the Burmese python (*Python molurus bivittatus*), an Asiatic snake species introduced to southern Florida, is able to kill adult humans by constriction (Rodda, Jarnevich and Reed, 2009). Particularly relevant for developing countries are IAS that can alter the water supply or cause a decrease in food disposability: the golden snail *Pomacea canaliculata* for example, which has been introduced into the Philippines in 1982 for food production, has become a major pest of rice (Halwart, 1994).

However, perhaps the greatest threat to human health from IAS is that of infectious diseases.

1.1.1 Invasions and infections

Besides IAS introductions, the breakdown in biogeographic barriers characterizing the last decades has facilitated the emergence/re-emergence of infectious diseases, defined by the American Centers for Disease Control and Prevention (CDC) as "diseases whose incidence in humans has increased in the past two decades or threaten to increase in the near future".

The number of emerging infectious disease events has increased significantly over time and notably, the 60% of these events are zoonoses, of which the vast majority originate from wildlife (Jones *et al.*, 2008).

Disease emergence is frequently associated with changes in the ecology of hosts, pathogens, or both (Daszak, 2000). In this sense, IAS not only facilitate the worldwide introduction of zoonotic agents (so-called "pathogen pollution", see Daszak, 2000), but, being characterized by rapid range expansion and dramatic increases in

local abundance, can also lead to dynamics that are exactly the precondition potentially triggering disease outbreaks (Hulme, 2014).

Despite this, forecasts of the risk of emerging diseases are neglecting the potential role of IAS (Hulme, 2014; Roy *et al.*, 2017), and it has been pointed out how IAS and emerging infectious diseases, despite sharing several similitaries, are studied by two branches of science (invasion science and epidemiology) that work in parallel rather than together (Ogden *et al.*, 2019). Ecologists and biologists, i.e. the main people traditionally involved in the study of IAS, have explored the topic of IAS pathogens mainly focusing on the impacts on biodiversity and the ecosystem functioning. As such, alien pathogens with documented negative effects on native species conservation, such as the squirrel poxvirus or the fungus *Batrachochytrium dendrobatidis*, which have contributed, respectively, to the decline of native European squirrel *Sciurus vulgaris* populations (Tompkins, White and Boots, 2003) and of several species of amphibians worldwide (Fisher, Garner and Walker, 2009), have been object of intense study. Ecologists focused on exploring the role of pathogens in the invasion process, through the formulation and testing of several concepts, in particular the enemy release hypothesis, which relates the success of IAS in the new environment to the loss of its natural co-evolved enemies (including pathogens) (Keane and Crawley, 2002; Torchin *et al.*, 2003), and the concept of "disease-mediated invasion", according to which invasive organisms gain an advantage on native competitors transmitting them pathogens affecting their fitness (Strauss, White and Boots, 2012).

Although this kind of studies allowed to gain relevant insights on the mechanisms and factors regulating the relationship between invasions and infections, unfortunately they still have found a scarce application in the health field, where researchers are giving limited attention to the understanding of the potential impacts of IAS (Hulme, 2014; Srebaliene *et al.*, 2019). Moreover, the taxonomic bias that has been found to characterize the invasion literature (Pyšek *et al.*, 2008) seems to reflect also in the study of alien species of health concern: a review analyzing the European research available on IAS of human health concern found that most articles were available for vascular plants and dipterans, with only a few concerning other taxa as mammal, ticks, amphibians, reptiles, and birds (Schindler *et al.*, 2015). A small number of organisms in particular appeared to dominate research, like the Asian tiger mosquito (*Aedes albopictus*), a vector of several pathogens of human health relevance, while other less known species are mostly neglected (Hulme, 2014; Schindler *et al.*, 2015).

IAS as hazards to public health

Despite the scarcity of available studies on the public health impacts of invasive mammal, ticks, amphibians, reptiles, and birds (Schindler *et al.*, 2015), empirical evidence suggests that several invasive species of these taxa may actually play a role in the circulation of zoonotic infectious disease. Several reptiles and amphibian species, including the much diffused *Trachemys scripta*, which was commercialized in Europe as pet until a few years ago, are asymptomatic carriers of salmonellosis (Ramsay *et al.*, 2007). Moreover, reptiles and amphibians imported for pet trade are

often parasitized by ticks, including the vectors of *Rickettsia* species affecting humans (Pietzsch *et al.*, 2006). In Japan, Goka, Okabe and Takano (2013) brought to attention how none of the national laws is regulating the import of reptiles' ectoparasitic ticks, and asked for the adoption of increased preventive measures.

European starlings in America are implicated in the transmission of *Escherichia coli* O157 to humans both directly (Ejidokun *et al.*, 2006) and indirectly, through livestock, contaminating cattle feed (Kauffman and LeJeune, 2011).

However, it is the lack of focus on mammal IAS that appears particular worryingly, as mammals -and rodents, carnivores, and ungulates in particular- have a recognized crucial epidemiological role in the transmission of zoonoses (Cleaveland, Laurenson and Taylor, 2001; Han, Kramer and Drake, 2016).

Rodents and carnivores are highly adaptable to new environments and often succeed in colonize both natural and urban areas (Bateman and Flaming, 2012; Capizzi, Bertolino and Mortelliti, 2014), thus increasing the possibilities for transmission of diseases among humans, pets and livestock and the risk of emerging infectious diseases (McFarlane, Sleigh and McMichael, 2012). Besides rats and mice *Rattus rattus, R. norvegicus and Mus musculus,* which are diffused worldwide and are known reservoirs of diseases of public health significance (Capizzi, Bertolino and Mortelliti, 2014; Capizzi *et al.*, 2018), there are rodent IAS far less considered from the health point of view that may as well represent a threat: two invasive squirrel species for example, *Tamias sibiricus* in France (Vourc'h *et al.*, 2007) and *Sciurus carolinensis* in the UK (Millins *et al.*, 2015), have found to be infected with the causal agent of Lyme disease, a chronic debilitating disease. The American mink *Neovison vison*, introduced as a fur animal in Russia and currently naturalized in many parts of Europe

(Birnbaum, 2013) has recently gained the attention of media as resulted to be highly susceptible to SARS-CoV-2, the causative agent of COVID-19 (Molenaar *et al.*, 2020), and spill-over events to humans have been documented, highlighting the need for further research to shed light on its possible role as reservoir of the disease (Manes, Gollakner and Capua, 2020; Oude Munnink *et al.*, 2021).

Finally, invasive ungulates can harbor a great number of zoonoses (Böhm *et al.*, 2007; Ferroglio, Gortázar and Vicente, 2011; Capizzi, Bertolino and Mortelliti, 2014; Capizzi *et al.*, 2018) such as echinococcosis and hepatitis E (Boadella, 2015), potentially exposing hunters, forestry workers and outdoor tourists to disease (Ruiz-Fons, 2015).

IAS as hazards to animal health: impacts on production, welfare and conservation

Invasive species health threats are not limited to humans, but extend to animals, both domestic and wild, with potentially serious consequences for livestock welfare and economy and biodiversity conservation.

Wild boar *Sus scrofa*, for example, can act as reservoirs for many important infectious diseases in domestic animals, such as African swine fever (Meng and Lindsay, 2009), a highly contagious disease with mortality rates that can be as high as 100%, causing severe socio-economic impact on the meat industry (Costard *et al.*, 2009). In China, where several outbreaks occurred during 2018, the disease reduced the national pig herd by half, with direct economic losses of US$ 141 billion (Berthe, 2020). The disease is currently representing a main concern in the northern hemisphere due to uncontrolled mobility of wild boars (Lange, Guberti and Thulke, 2018).

In Europe, the invasive signal crayfish *Procambarus clarkii* acts as reservoir for *Aphanomyces astaci*, a fungus responsible for crayfish plague, which have caused estimated economic losses to fish farms in Europe of over 53 million/year (European Union, 2014a), and the extinction of native crayfish populations (Souty-Grosset *et al.*, 2016).

Invasive species can harbor pathogens of concern for native wild species (Daszak, 2000), especially if native species live in small populations and restricted areas. American minks *N. vison*, for example, can transmit Canine distemper virus (CDV), a highly lethal morbillivirus representing a recognized threat for several species of conservation relevance such as the gray wolf *Canis lupus* (Almberg *et al.*, 2012), and the African lion *Panthera leo* (Packer *et al.*, 1999). A study suggested that in Chile, invasive minks acted as bridge hosts for CDV between domestic dogs and threatened river otters (*Lontra provocax*), two species that in natural conditions would not have been connected (Sepulveda *et al.*, 2014).

Whereas some aspects of public health connected to biological invasions, such as mosquito-borne diseases, are managed, others, including potential vertebrate hosts, are far less addressed. This results in a lack of understanding of the real magnitude of the issue, which inevitably reflects in a lack in strategies to prevent the possible negative impacts on health.

2. Better safe than sorry: estimating risks in animal health

2.1 From science to policy: the risk analysis process

A risk, as defined by the Compact Oxford English Dictionary of Current English, is the possibility that something unpleasant will happen, and it composes of two components: the likelihood of occurrence and the magnitude of consequences of a specified hazard being realized.

The need to prevent the occurrence of events that could lead to unacceptable levels of risk led to the birth of risk analysis, a systematic process intended to support rational decision-making and policy-making in the face of uncertainty, through the logical use of the available scientific evidence (Jakob-Hoff *et al.*, 2014).

The risk analysis field emerged in the late 20th Century, when people belonging to several disciplines, including engineering, economy, finance, public safety and occupational health, begun to standardize methods to assess and predict risks in their field (Jakob-Hoff *et al.*, 2014). However, it was only relatively recently that the process begun to be applied to the animal health sector, where, until then, veterinarian officers were still basing their decisions on common sense and experience (Jakob-Hoff *et al.*, 2014). In particular, the field has evolved after the 'Agreement on the Application of Sanitary and Phytosanitary Measures' entered into force with the establishment of the World Tread Organization (WTO) in 1995, which established that any restrictive measures adopted by WTO members because of sanitary reasons must be appropriate and justified by the outputs of recognized methodologies for the assessment of the risk to human, animal or plant health. After risk analysis acquired the role of 'instrument of guarantee' against protectionism, the need for

standardized references guiding the process emerged (Crotta, 2014), and relevant international organizations begun to develop ad-hoc frameworks. Two, in particular, are of interest in the animal health sectors, and are applied according to the risk question under consideration:

- *The World Organisation for Animal Health (OIE) risk analysis framework*

Developed by the World Organisation for Animal Health (World organisation for animal health, 2008), this model is primarily intended as a tool for import risk analysis, which aims to answer the question related to the possible magnitude of disease risk posed by the importation of animals and animal products. The process consists of four steps: Hazard Identification, Risk Assessment, Risk Management and Risk Communication. The risk assessment step is further divided in: Entry assessment, Exposure assessment, Consequence assessment, and Risk estimation.

- *The Codex Alimentarius risk analysis framework*

The model developed by the Codex Alimentarius Commission of the Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) (WHO and FAO, 2008) applies specifically to microbiological food safety. It has been developed to answer the question related to the maximum amount of a substance (or pathogen) to which a person should be allowed to be exposed from a particular source, and it is thus a regulatory tool for setting allowed, acceptable or tolerable levels of contaminants and pathogens in food. The process consists of three

steps: Risk Assessment, Risk Management and Risk Communication. The risk assessment step is further divided into four steps: Hazard identification, Hazard characterization, Exposure assessment, and Risk characterization.

2.2 Wildlife disease risk analysis

Risk analysis is now an accepted basis for establishing international trading standards on the import of production animals and animal derived products, as well as the acceptable levels of contaminants in food products. However, its application to wildlife remains still in its infancy; the first framework for wildlife disease risk analysis (DRA), developed starting from the OIE framework, was published in 2014 (Jakob-Hoff *et al.*, 2014), with the aim to encompass the special features associated with disease risk analysis as it is applied to wildlife. Working with wildlife poses indeed relevant challenges, as there are multiple variables influencing the introduction, establishment and spread of infectious agents among populations (of single or multiple species), data on disease in wildlife populations are often limited, and resources like time, money, equipment, people and relevant expertise are often in short supply (Jakob-Hoff *et al.*, 2014).

The steps of wildlife DRA are the following (Jakob-Hoff *et al.*, 2014):

- Problem description: outline of the background and the context of the problem, and identification of the goal of the DRA process;
- Hazard identification: identification of all possible health hazards of concern and establishment of criteria for ranking the importance of each hazard;
- Risk assessment: assessment, for each hazard of concern, of the likelihood of introduction into the environment of concern (release assessment), the likelihood

that the species of interest is exposed to the hazard (exposure assessment), and, if the risk of exposure is significant, of the consequences (biological, environmental, social, economic) and related magnitude of the entry, establishment or spread of the hazard.

- Risk management: review of potential risk reduction or management options and evaluation of their likely outcomes. On this basis, decisions and recommendations can be made to mitigate the risks associated with the identified hazards;
- Implementation and review: formulation of an action and contingency plan and establishment of a process and timeline for the monitoring, evaluation and review of risk management actions;
- Risk communication (throughout all the analysis steps): involvement of a wide group of technical experts, scientists and stakeholders to maximize the quality of analyses and the probability that recommendations arising will be implemented.

2.2.1 Disease risk assessment

Disease risk assessment, defined as "the process of estimating the likelihood of a pathogenic agent (from any defined source) entering, establishing or spreading in a country, zone or population and its accompanying impact(s) on animal or human health, the environment or the economy" (Jakob-Hoff *et al.*, 2014), is the step of risk analysis involving the practical estimation of the risk, hence the one of scientific pertinence (Crotta, 2014), and provides the basis for prioritizing hazards to determine

whether or not risk prevention or mitigation measures are needed. The methodology used to assess risks can be qualitative or quantitative, depending on the aims of the analysis and on the quality/amount of available data.

While both methodologies foresee the identification of the risk pathway, i.e. the chain of steps required for the risk to occur, differentiating release, exposure and consequence, there are significant differences in the ways risks are defined and combined.

Qualitative methods use discrete levels to describe the probability of the unwanted event to occur and the magnitude of the consequences, such as 'high', 'medium' or 'low'. Such methods are extensively used as a first approach in routine decision-making processes, when data are insufficient, for example for emerging risks, or when time is limited, as in case of health emergencies (Crotta, 2014). In wildlife DRA, qualitative analysis is the most common approach, as data rarely make quantitative assessments possible (Jakob-Hoff *et al.*, 2014).

Quantitative methods use mathematical models to describe the events along the risk pathway, and as such, they require mathematical expertise, time and a considerable amount of data. Mathematical models can be deterministic, when both the inputs and outputs are expressed as single numbers, or probabilistic, when the factors involved in the model are described as probability distributions. Probabilistic approaches are normally more adapt to describe the variability and the uncertainty of biological events. When adopting probabilistic approaches, the probability of an unwanted event is quantified by using simulation techniques (e.g. Monte Carlo) (Crotta, 2014).

Risk assessment, through the reproduction of the real system, represents a transparent instrument to identify the events posing the highest risks and, through the assessment of the uncertainty surrounding risk estimates, the major knowledge gaps, thus that risk managers and policy makers can direct resources and legislation where they are most needed.

Despite the recognition that IAS risk towards human health should be evaluated through appropriate risk assessments (European Union, 2014b) and despite a multitude of risk assessment procedures to evaluate IAS environmental impacts have been developed (Hulme, 2014; Srebaliene *et al.*, 2019), a standardized ad-hoc method to prioritize animal IAS based on their disease risk is still lacking.

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Outline of the Thesis

Despite the increasing attention received in recent years by biological invasion and emerging infectious diseases, and their many point of contacts, these two fields still lack of a common ground, and the potential disease risks of animal IAS remain overlooked.

The main aim of this thesis is therefore to cover this gap by setting the ground for a new "invasion epidemiology" field, and this has been done through two main steps: the review and analysis of both the mechanisms underlying IAS disease risk and the information available in literature on IAS pathogens, and the development of a standardized qualitative disease risk assessment method, applicable to different geographic contexts, to assess the risk of mammal IAS to impact on human and animal health.

As a first step, I reviewed the existing biological and ecological literature on IAS to define the main mechanisms by which animal IAS may affect disease risk, while at the same time raising awareness in people working in the animal and public health field on the health threat IAS may represent and on the need to develop specific risk assessment tools (**Chapter 2**). Then, as information on IAS pathogens was not systematized, I systematically reviewed the literature on the infectious agents of the main mammal IAS of European Union concern in order to evaluate the amount and quality of the information available. This allowed to gain insights on the level of knowledge we currently have on IAS infectious agents and to better characterize the existing gaps (**Chapter 3**). Considering that the existing knowledge gaps would have resulted in strong limitations in informing a disease risk assessment procedure, I

developed a methodology based on expert opinion. This risk assessment methodology (**Chapter 4**) has been designed to be applicable to different geographical contexts and mammal IAS species, and allows to assess the disease risks that established populations of these species would pose/pose (if already established in the area) to local human and animals populations, trough the possible transmission of new or local pathogens of recognized health significance. As this methodology foresees the combination of qualitative estimates related to multiple risk pathways, and no standardized method to perform this kind of operation was available in the qualitative risk assessment literature, I developed a method to combine qualitative estimate allowing for an increase of risk, with the aim to reduce the inherent subjectivity of qualitative methodologies (**Chapter 5**). Lastly, a synthesis of the main thesis findings is provided, and implications and possible directions for future research are discussed (**Chapter 6**).

CHAPTER 2

Invasive alien species and disease risk: an open challenge in public and animal health

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PLoS Pathogens (2020) 16(10): e1008922

Why we should care about invasive alien species from a health perspective

The anthropogenic movement of pathogens into new geographic locations or host species, so-called "pathogen pollution" (Daszak, 2000), is one of the main threats to human and animal health in a globalized world.

Since the majority of zoonotic emerging diseases originate from wildlife (Jones *et al.*, 2008), as recent outbreaks, like SARS-CoV-2, Nipah or Chikungunya point out, particular attention should be paid to wild animals' translocations, which represent a potential driver of change in pathogen ecology and distribution (Daszak, 2000).

Invasive Alien Species (IAS) are species of animals, plants, fungi or microorganisms translocated by humans into environments outside their natural range, in which they establish and spread, negatively affecting local ecosystems' dynamics. They are characterized by rapid reproduction and growth, high dispersal ability and high adaptability to new conditions, thus often out-competing native organisms in their introduced range, and have been recognized as one of the main causes for biodiversity loss globally (Bellard, Cassey and Blackburn, 2016). Some well-known examples of IAS include the south-American coypu *Myocastor coypus*, invasive in North America, Europe, and Asia, where it causes both environmental and economic impacts consuming aquatic vegetation and undermining riverbanks (Global Invasive Species Database, 2020), and the eastern-Asiatic Brown Marmorated Stink Bug *Halyomorpha halys*, a successful global invader causing severe economic damages to agricultural crops (Leskey and Nielsen, 2018).

Besides affecting biodiversity conservation and economy, IAS, as translocated species, may promote pathogen pollution in the invaded area leading to the emergence of diseases (Daszak, 2000; Crowl *et al.*, 2008; Conn, 2014). It would thus be fair to expect animal IAS to be the focus of intense study by epidemiologists with regard to their disease risk towards native animals (both wild and domestic) and humans, as most of them thrive in anthropogenic environments, potentially increasing the risk for zoonotic pathogen emergence (Hulme, 2014).

Within the field of invasion ecology there has been a wide interest in exploring the relationships between invasions and infections during the last decades. Researchers focused in particular in understanding how parasites (or the lack of them) may facilitate or hamper the invasion process (Colautti *et al.*, 2004; Dunn *et al.*, 2012; Strauss, White and Boots, 2012; Blackburn and Ewen, 2017), how co-introduced parasites may themselves succeed in becoming invasive (MacLeod *et al.*, 2010; Lymbery *et al.*, 2014; Blackburn and Ewen, 2017), and explored the effects that IAS may have on native parasites dynamics (Telfer *et al.*, 2005; Dunn, 2009; MacLeod *et al.*, 2010; Blackburn and Ewen, 2017). However, outside the invasion ecology field, IAS have yet to gain attention among people working in the fields of animal and public health, and the concepts explored in the ecological context cannot always find application in the development of health initiatives aimed at protecting public and animal health. For example, empirical research on IAS pathogens, which would be needed to assess the risk of infectious disease emergence, is skewed towards a few species (e.g. vector species like the tiger mosquito *Aedes albopictus*) or towards selected pathogens known to harm biodiversity conservation, while a

global vision of IAS-associated health threats is still not available (Hulme, 2014; Schindler *et al.*, 2015; Roy *et al.*, 2017; Peyton *et al.*, 2019).

In this context, it is urgent to raise awareness in people working in the fields of animal and public health of the need to consider IAS as a health threat. To this aim, we provide here an overview of how animal IAS may affect local disease dynamics both directly and indirectly, i.e. acting as pathogen hosts or disrupting the recipient ecosystem structure, through real-case examples from the ecological literature, and, in the last paragraph, we propose future initiatives aimed at improving our capacity for targeted actions towards the IAS most likely to threaten human and animal health, calling for an increased involvement of people working in the fields of animal and public health in a new invasion epidemiology field.

IAS as sources of new pathogens

IAS may host pathogens that are absent in the area of release and cause their establishment and subsequent spillover to local species, possibly resulting in an increase of disease risk for humans, domestic animals and native wildlife.

The north-American raccoon *Procyon lotor*, for example, introduced to Central Europe *Baylisascaris procyonis* (Rentería-Solís *et al.*, 2018), a nematode causing *larva migrans* syndromes potentially inducing severe central nervous system disease in humans (Fig 1A). Introduction to Europe of north-American crayfishes *Procambarus clarkii* infected with the fungus *Aphanomyces astaci* caused huge economic losses to fisheries, being the pathogen lethal to native crayfishes (Gherardi, 2007). Similarly, squirrelpox virus, introduced to the UK along with the American

eastern grey squirrel *Sciurus carolinensis,* is significantly contributing to the increased mortality of native red squirrels *Sciurus vulgaris* (Tompkins, White and Boots, 2003).

However, while pathogen co-introductions occur over a wide range of parasite and host taxa (Lymbery *et al.*, 2014), some pathogens are lost during the invasion process (Torchin *et al.*, 2003): for example, there is no evidence for Poxvirus in Italian grey squirrel populations (Romeo *et al.*, 2019). Pathogen loss may be due to the absence of the pathogen in the individuals of the founding populations, or to its inability to survive to translocation or establish in the area of release. The outcome depends on several factors related to the IAS (e.g., founding population origin), the pathogens (e.g., host specificity) and the area where the species is released (e.g., environmental conditions, presence and density of local hosts) (MacLeod *et al.*, 2010). As shown by a study on ectoparasites of introduced birds, factors related to transmission efficiency, such as the number of host introduced and host longevity, are likely to play a major role (MacLeod *et al.*, 2010).

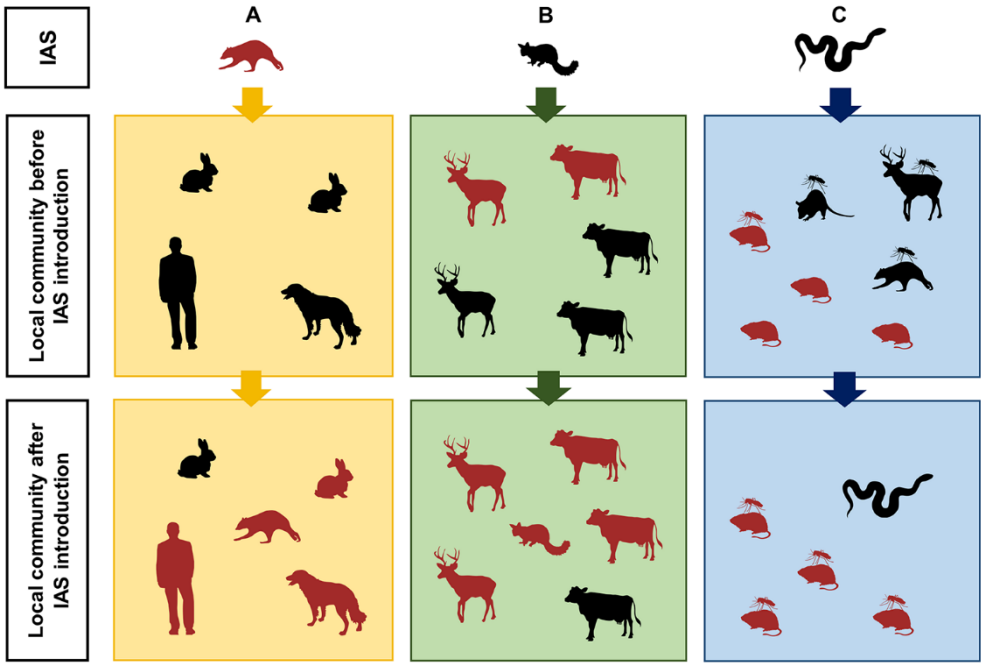

Figure 1 - Mechanisms through which invasive alien species may increase disease risk: real-case examples. Dark red silhouettes represent infected hosts, black silhouettes uninfected hosts.
(A) IAS as sources of new pathogens: the North-American raccoon Procyon lotor *introduced the nematode* Baylisascaris procyonis *into central European countries. Raccoon is* B. procyonis *definitive host and sheds parasite eggs through feces, contaminating the environment. Small mammals and birds may serve as paratenic hosts, while domestic dogs, although rarely, may act as alternative definitive hosts. Humans, which acquire the infection as accidental hosts, can develop severe symptoms, caused by larvae migration to tissues.*
(B) IAS as amplifiers of local pathogens: the invasive Australian possums Trichosurus vulpecula *became the main reservoir host for bovine tuberculosis in New Zealand. Despite* Mycobacterium bovis *was introduced to New Zealand via cattle in the 1800s and possums in the 1850s, the disease emerged in possum populations only in the 1970s, in locations occupied by wild deer, when decapitation of deer was a common hunting practice. Currently, intensive possum control actions, which cost to the country about $NZ50 million per year, allowed to obtain huge reductions in the number of infected cows and deer, but New Zealand is still not free from the disease.*
(C) Indirect mechanisms by which IAS can disrupt local infection dynamics: in Florida, invasive pythons Python bivittatus *reduced the abundance of several large and medium-sized mammals, indirectly causing the redirection of the mosquito vectors for the zoonotic Everglade virus from low competent hosts, like deer, raccoons and opossums, to the main reservoir host, the hispid cotton rat* Sigmodon hispidus*. Further research is needed to assess if the increased abundance of infectious vectors corresponds to an increase of disease risk for local human populations*

IAS as amplifiers of local pathogens

An increase of local disease risk may also occur if the introduced IAS is susceptible to, and able to transmit, local pathogens. Pathogens acquired by IAS may be amplified and possibly spill back to humans and local species (Kelly *et al.*, 2008).

A case in point is the Australian brushtail possum, *Trichosurus vulpecula,* in New Zealand (Fig 1B). Invasive possums probably became infected with *Mycobacterium*

bovis, the causal agent of tuberculosis in cattle, from wild deer, after the beginning of commercial deer hunting in 1960. Currently, they are the most important maintenance host for bovine tuberculosis, supporting higher transmission rates compared to local species and, being sympatric with cattle, providing interface for transmission between livestock and forest residents (Nugent, Buddle and Knowles, 2015). Another case is represented by invasive raccoon dogs *Nyctereutes procyonoides*, which may amplify rabies circulation in Eastern Europe or cause its re-emergence in currently rabies-free countries (Singer *et al.*, 2009).

IAS competence for pathogen transmission plays a major role in defining the outcome of pathogen acquisition, and, as the possum-tuberculosis case exemplifies, it is the result of both IAS-pathogen interaction (e.g., IAS susceptibility, period of communicability and pathogen excretion rate) and IAS behavioral patterns (e.g., habitat, home rage extension, intra and inter-specific contact rates).

Based on IAS competence, the acquisition of a local pathogen may even lead to the reduction of disease risk (the so-called dilution effect, Keesing, Holt and Ostfeld, 2006) or to no consequences at all. For example, in Ireland the invasive bank vole *Myodes glareolus* has been found to divert fleas from the native wood mice *Apodemus sylvaticus*, which is a more competent host for *Bartonella* spp. (Telfer *et al.*, 2005). However, the identification of the contexts in which a dilution effect may occur is still highly debated in ecology, as it strongly depends on local host species diversity and on the interactions occurring between the species involved in the transmission cycle (Keesing, Holt and Ostfeld, 2006).

Indirect mechanisms by which IAS can disrupt local infection dynamics

Introduced species may disrupt local infection dynamics also indirectly, i.e. non-acting as pathogen hosts but through competitive and trophic interactions with native species or modification of local habitats, thus altering the abundance and/or contact rates among local host species, parasite infective stages or vectors.

In southern Florida, the invasive python *Python bivittatus* caused the decrease of several mammal species, inducing the local mosquito vector of zoonotic Everglades virus to feed almost exclusively on the virus' main reservoir host, the hispid cotton rat *Sigmodon hispidus*, potentially leading to an increase in pathogen circulation (Fig 1C) (Hoyer *et al.*, 2017). An example of habitat alteration is given by the activity of invasive feral pigs *Sus scrofa* on the island of Hawaii: they create wallows and cavities in tree fern trunks improving habitat suitability for mosquito vectors for avian malaria *Plasmodium relictum* (LaPointe *et al.*, 2016), one of the main threats to native Hawaiian forest birds' conservation.

Again, IAS indirect effects on local infection dynamics are highly context-dependent, and mechanisms presented so far may act in concert, producing unpredictable outcomes. In Scotland and Northern England, for example, the invasive grey squirrel has been found to harbor several local strains of *Borrelia burgdorferi* (Millins *et al.*, 2015). However, in those areas, grey squirrels are also causing the decline of another competent host for *B. burgdorferi*, the red squirrel, and the effect of these concurring mechanisms on human Lyme disease risk remains unknown (Millins *et al.*, 2015).

A call for action: from invasion biology to invasion epidemiology

During the last centuries more than 16.000 IAS introduction events have been recorded worldwide, and this number still presents an increasing trend (Seebens *et al.*, 2017). In such context, the identification of those species deserving priority attention, based on their actual and potential impacts, is essential to support decision-making (McGeoch *et al.*, 2016). Several tools to inform preventive and management actions on animal IAS, including horizon scanning protocols, risk assessments and impact assessments, have been developed in the last years (see Roy *et al.*, 2018 for a recent review), but the majority of them focuses on environmental impacts, not specifically considering disease emergence risks in humans and local animal populations (Essl *et al.*, 2011; Srebaliene *et al.*, 2019). Some authors have called for a greater attention on the potential health risks posed by biological invasions (Conn, 2014; Hulme, 2014; Roy *et al.*, 2017), highlighting the need for a better integration between biological and health sciences, surveillance actions and coordinated policies. We support their appeal, arguing that an increased awareness of people working in the fields of animal and public health on the risks concerning biological invasions and their consequent involvement in the invasion biology field is the first step towards a complementary invasion epidemiology field. Such field would be integrated with invasion ecology, but more specifically aimed at the prevention of the emergence of diseases in human and animal populations consequent to IAS introduction and establishment. To this aim, we propose some initiatives that should be addressed by future research work.

A first major constraint in addressing the issue of disease emergence connected to IAS is given by the lack of comprehensive data on pathogens affecting IAS. In this sense, we recommend the gathering in ad-hoc databases of all the available information on IAS pathogens affecting human and animal health, including their geographical distribution and prevalence in IAS populations, in both native and introduced ranges.

It would also be advisable to improve our understanding of the key epidemiological events and factors driving the emergence of infectious disease following IAS establishment, for example through ex-post analyses on the already established IAS. In particular, as the emergence process of a disease is composed of several stages (introduction in a new area/host population, establishment and spread) (Jeschke, Keesing and Ostfeld, 2013; Lymbery *et al.*, 2014; Dunn and Hatcher, 2015), the key factors involved in the process and related to IAS biology, pathogenic features and the biotic and abiotic components of the area of release should be identified for each of these stages.

We also suggest to urgently direct research efforts at developing transparent and flexible tools able to prioritize IAS based on the risk of transmitting pathogens with the potential to impact the health of humans, production animals and native wildlife. Such tools could be based on the framework of the OIE/IUCN disease risk analysis for wildlife and re-adapted to account for the main mechanisms through which alien species may affect local health, in particular the introduction of new pathogens and the acquisition and spread of local ones. The lack of data on IAS pathogens is certainly an obstacle in underpinning in-depth risk assessments (Roy *et al.*, 2017), in particular quantitative ones. However, a simple and transparent qualitative disease

risk assessment procedure would enable to prioritize the empirical research needed to cover these knowledge gaps, while at the same time guiding local health administrators in the allocation of resources for management and preventive actions towards IAS. The issue related to irregular data availability could be partially overcome, as a first step, by eliciting opinions from experts.

Finally, awareness and action will be influenced by, and need to consider, the wider public perspective, not just researchers and institutions. Initiatives aimed at sensitizing citizens about the health threats of IAS will be needed to promote responsible behaviors when crossing borders and to improve the general public attitude towards IAS control and eradication programs.

All the suggested initiatives, to be successful, necessitate a stronger connection between ecologists, biologists and other people working in the fields of animal and public health and beyond. Only through wider collaboration and dialogue will the potential health impacts of biological invasions be fully appreciated and, perhaps, ameliorated.

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CHAPTER 3

When knowing to not know is better than not knowing at all: exploring gaps in the research on Invasive Alien Specie infections

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Manuscript

Introduction

Invasive Alien Species (IAS), or species introduced by humans in environments outside their natural geographic range, are a recognized environmental issue harming the conservation of native species worldwide and causing billions of dollars in damages every year (Pimentel *et al.*, 2001).

During the last years, governments and international organizations have put in place specific legislation and plans to deal with the IAS issue, like the European Reg. 1143/2014 and related implementing acts, which issue a list of priority species on which to direct preventive and management actions (European Union, 2014, 2016, 2017, 2019). The high and worryingly increasing global number of introduction events (Seebens *et al.*, 2017) makes indeed the identification of the species at highest risk to cause adverse impacts indispensable to guide preventive and management actions (McGeoch *et al.*, 2016). As a consequence, risk assessments, impact assessments and horizon scanning procedures are now the standard approaches used to identify species requiring priority attention (Roy *et al.*, 2018). The species included in the European list of Union concern, for example, should be defined and updated according to the output of specific risk assessments aimed at estimating IAS risks towards "biodiversity, economy and human health".

Among the potential impacts of IAS there is the one connected to their possible role in the emergence or re-emergence of infectious diseases (Hulme, 2014; Chinchio *et al.*, 2020): IAS may indeed alter the infection dynamics existing in the areas where they are released through several mechanisms, and in particular acting as host or vectors of new or endemic pathogens relevant to public and animal health.

The need to consider animal IAS from a public health perspective, and to assess their disease risk through ad-hoc risk assessment methodologies has been highlighted during the last years (Hulme, 2014; Chinchio *et al.*, 2020). However, health-related aspects continue to be scarcely considered in the development of risk assessment methodologies in the field of invasion biology, which remain primarily devoted to the assessment of environmental impacts (Srebaliene *et al.*, 2019).

Disease risk assessments (Jakob-Hoff *et al.*, 2014) aimed at predicting health threats associated with IAS establishment, and in particular the possible role of IAS as introducers of new pathogens or amplifiers of endemic ones, require baseline data on the pathogens harbored by IAS, but these data are suspected to be patching and lacking (Hulme, 2014). As a matter of fact, while diverse global databases include information about biological features and ecological impacts of IAS, data on their pathogens are not yet systematized (Hulme, 2014), preventing us from knowing what kind of information is available to inform possible risk assessment procedures and from making meaningful inference about IAS-related health risks.

To gain insights in the actual availability and quality of information on IAS pathogens, in the current paper we systematically reviewed the scientific literature related to the pathogens of the eleven invasive mammal species included in the list of IAS of European Union concern. We focused on mammals as they are the most implicated in the transmission of zoonotic diseases (Woolhouse, Haydon and Antia, 2005; Jones *et al.*, 2008). The enlisted mammal species include species of rodents, carnivores and ungulates, all orders with an established role as zoonotic reservoirs (Cleaveland, Laurenson and Taylor, 2001; Han, Kramer and Drake, 2016).

In particular, we investigated the drivers of the research intensity on the topic of IAS infections, IAS pathogen species richness (i.e., the total number of pathogens hosted by a species), and the pooled prevalence of selected pathogens of public and animal health significance. Results are discussed in order to highlight the existing limitations and data gaps, and possible solutions are suggested.

Methods

Systematic review of the literature

We run a systematic review in order to investigate the infectious agents affecting the following mammal IAS of Union concern (European Union, 2014): carnivores *Herpestes javanicus*, *Nasua nasua*, *Nyctereutes procyonoides* and *Procyon lotor*, rodents *Callosciurus erythraeus*, *Myocastor coypus, Ondatra zibethicus, Sciurus carolinensis*, *Sciurus niger* and *Tamias sibiricus*, and the ungulate *Muntiacus reevesi*. In particular, we collected information about virus, bacteria, protozoa, helminths and ecto-parasites hosted by these species.

This systematic review followed the Cochrane and PRISMA Group guidelines (Moher *et al.*, 2009; O'Connor and Sargeant, 2014; Higgins *et al.*, 2019). Literature search was carried out using the following databases: PubMed, Scopus, Web of Science Core Collection, Cab Abstracts and Global Health. Research strings were developed using different combinations of words and Boolean operators in order to maximize the number of results and were adapted for each database (see **Supplementary Material S1**). Literature results were checked for duplicates, which were removed, and title-abstract screening was performed using the metagear package

in R (Lajeunesse, 2016), according to the inclusion/exclusion criteria described below. The remaining articles were screened in full text.

Studies eligible for inclusion in full-text review included primary research articles in English language reporting cases of infection caused by virus, bacteria, protozoa, helminths or ecto-parasites. The following articles were excluded from the systematic review: experimental studies, studies on non-infectious pathogens, studies where the causal agent was not clearly identified or not identified to genus, studies on hosts other than the IAS, studies reporting non-original data (i.e. reviews and data already published elsewhere) and theoretical epidemiology studies. No time limit was posed, but studies not accessible online were not considered in the analysis. In those cases, data were extracted from abstracts when possible. The list of the articles included in the analyses for each species is available in **Supplementary Material S2**.

For each research article, we collected information related to the host species, the pathogen species, and the host samples analyzed, and stored them in relational databases in Microsoft Access.

Each pathogen was classified based on its taxon. The name of each pathogen was updated based on current nomenclature databases, referring to the International Committee on the Taxonomy of Viruses for virus nomenclature, and to the National Center for Biotechnology Information Taxonomic database for the nomenclature of other pathogens. With regard to the host species, we collected information on the number of individual hosts sampled, the number of positive cases, the proportion of positive cases on the number of hosts sampled (if available), the sampling

locality and the methods used for pathogen identification. For each sampling locality, besides collecting data related to the country, it was further specified if the sampling area belonged to the natural geographic range of the IAS (i.e., area of origin), or to areas where the species has been introduced (i.e., area of introduction).

From these data, we obtained, for each pathogen taxon (bacteria, virus, protozoa, helminths and ectoparasites) of each host species: the number of pathogens (hereafter defined as "pathogen species richness"), the number of research articles, and the number of hosts sampled. These three outputs were also computed for each of the two areas of the IAS (area of origin and introduction), thus that each host species resulted to have ten data records: five for the taxa in the area of origin of the IAS, and five for the taxa in the area of introduction of the IAS.

Research intensity analyses

To explore the main factors affecting the amount of the investigations carried out on IAS pathogens, we fitted two generalized linear mixed models considering the host species, the pathogen taxon and the area of study as explanatory variables, and respectively the number of articles and the number of hosts sampled as response variables. Due to the highly skewed distribution of data, we used a negative binomial error structure, which better fitted data. To account for the repeated measures within each host species and pathogen taxon, these two factors were also included in the model as random factors.

Pathogen richness analyses

In order to identify factors contributing to the observed pathogen species richness, we analyzed the effect of the number of articles published and the number of hosts sampled on the observed pathogen species richness fitting a GLMM with negative binomial error structure. Pathogen taxa, the area of study and the host species were included as additional covariates. In particular, the interaction between pathogen taxa with the number of articles and the number of hosts sampled was considered in order to test their different effect on pathogen richness. Host species and pathogen taxa were considered as random factors in order to account for the repeated measures.

To estimate the level of knowledge we currently have on IAS pathogens, we applied approaches used in diversity ecology to estimate the true species richness in an area based on the samples taken, i.e. species accumulation curves (Dove and Cribb, 2006; Gotelli and Colwell, 2011; Chao *et al.*, 2014). In parasitology, species accumulation curves are applied to estimate the parasite species richness based on the parasite species found in host samples. Species accumulation curves chart the accumulation of new species recovered relative to a measure of the sampling effort. In this study, as a measure of the sampling effort, we considered the factor among the number of articles or the number of hosts sampled that was found to better predict pathogen richness as a result of the previous research intensity analyses. The curve asymptote, i.e. the total species richness estimated to characterize the species based on the samples, is obtained through the Chao2 estimator, which uses the frequency of the rarest species (i.e. the species found less frequently) in the samples to estimate the frequencies of undetected species (S_{est} in Equation 1)

(Chao, 1987). More specifically, the observed pathogen species richness S_{obs} is corrected by adding a term based on the number of parasite species represented in only one sample (singletons, Q1 in Equation 1) and in two samples (doubletons, Q2 in Equation 1).

Equation 1 - Chao2 Richness Estimator

$$Sest = Sobs + \frac{{Q_1}^2}{2Q_2}$$

Curves have been computed for the taxa of helminths using the iNEXT package in R (Hsieh, Ma and Chao, 2016), which is based on the statistical sampling models described in Colwell *et al.*, 2012.

Meta-analysis of pathogens of public and animal health significance

In order to evaluate the accuracy of information for the pathogens that are known to affect IAS, we identified the pathogens of public and animal health significance among the ones obtained through the systematic review, and carried out a meta-analysis of prevalence (Barendregt *et al.*, 2013) for each of them, where possible.

Pathogens of health significance are defined here as those included in the following EU legislation/institutional lists (see **Supplementary Material S3** for the complete list of pathogens):

- Decision (EU) 2018/945 on the communicable diseases and related special health issues to be covered by epidemiological surveillance (pathogens of public health significance);

- OIE list of notifiable diseases and Regulation EU 2016/429 (European Animal Health Law) (pathogens of domestic animal health significance);

- List of non-notifiable diseases developed by the OIE Working Group on wildlife diseases (pathogens of wildlife health significance).

Case reports/case series, studies on animals not belonging to wild populations (i.e. pets, captive wild animals), and studies with data inadequate to obtain the prevalence of the pathogen at species or genera level (e.g., studies reporting prevalence in broad categories of host/parasite individuals like "nematodes" or "squirrels") were not included in the meta-analysis. With regard to studies' quality, we decided to not include in the meta-analysis studies with a sample size lower than 10 animals and with evident sampling biases, i.e. studies on symptomatic animals/animals whose dead was attributable to the infectious disease object of study.

The meta-analyses were performed using the Excel add-on MetaXL (Barendregt and Doi, 2015) to estimate the pooled prevalence for each species pathogen. Due to the high heterogeneity among studies included in the analysis related to both the study setting and the methodologies used, a random-effect model was applied (Borenstein *et al.*, 2010). Data were transformed with the double arc-sin transformation (Barendregt, *et al.* 2013). When possible, a subgroup meta-analysis (Borenstein and Higgins, 2013) per area of study has been performed to obtain the pooled prevalence for both the area of origin and the areas of introduction of the IAS.

Results

Systematic review of the literature

The number of studies analyzed in each stage of the systematic review process for the investigated species is summarized in the PRISMA flow in Figure 1. Bibliographic searches, after the studies were screened for eligibility, identified a number of articles significantly different between host species (Table 1), with *P. lotor* being the most sampled (each pathogen taxon of the raccoon had a mean of 114.6 articles) and *H. javanicus* the lowest (each pathogen taxon of the mangoose had a mean of 2.2 articles). The number of articles did not differ among pathogen taxa, while it was significantly higher in the area of origin (each pathogen taxon of each host had a mean of 15 articles) respect to the area of introduction (each pathogen taxon of each host had a mean of 6.9 articles) (Table 1).

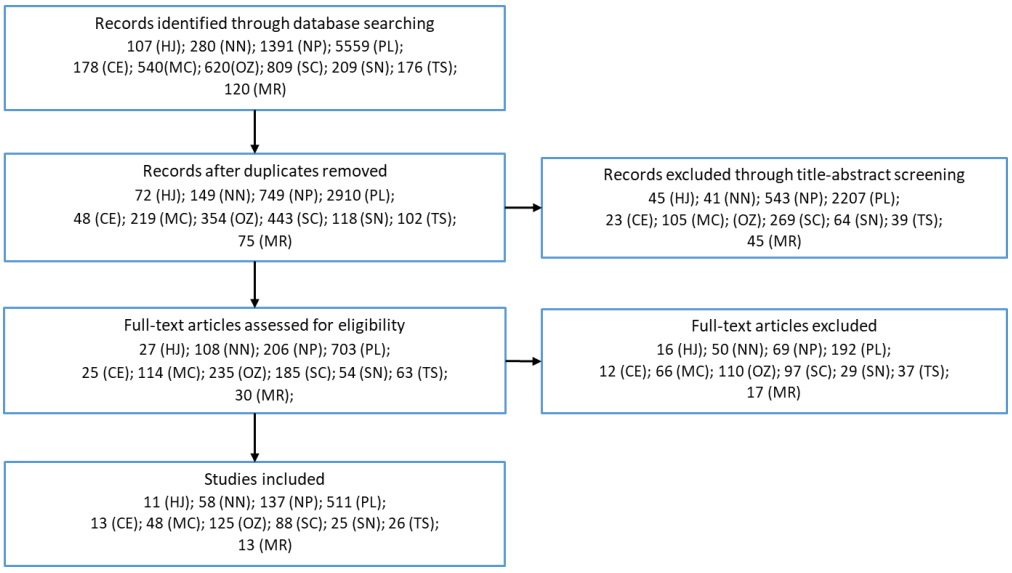

Figure 1 - PRISMA flow describing the systematic review process for the eleven IAS.
HJ=Herpestes javanicus, *NN*=Nasua nasua, *NP*=Nyctereutes procyonoides, *PL*=Procyon lotor, *CE*=Callosciurus erythraeus, *MC*=Myocastor coypus, *OZ*=Ondatra zibethicus, *SC*=Sciurus carolinensis, *SN*=Sciurus niger, *TS*=Tamias sibiricus, *MR*=Muntiacus reevesi.

The number of hosts sampled did not differ among areas or among pathogen taxa, while it was different among host species, with *P. lotor* being the most sampled (each pathogen taxon of the raccoon had a mean of 22,000 hosts sampled) and *M. reevesi* the lowest (each pathogen taxon of the muntjac had a mean of 19 hosts sampled) (Table 2).

Table 1 - Results of Generalized Linear Mixed Model analyzing factors explaining the number of research articles

Variable	d.f.	χ^2	P value
Area	1	10.2	0.001
Pathogen taxon	4	7.6	0.104
Host species	10	214.2	<0.001

Table 2 - Results of Generalized Linear Mixed Model analyzing factors explaining the number of sampled animals

Variable	d.f.	χ^2	P value
Area	1	0.1	0.954
Pathogen taxon	4	2.4	0.659
Host species	10	107.7	<0.001

Pathogen species richness analyses

The number of pathogens extracted from the articles (i.e. the observed pathogen species richness) ranged from a minimum of 11 (*M. reevesi*) to a maximum of 345 (*P. lotor)* (Table 3), with 5/11 host species having an observed pathogen species richness lower than 50 (*M. reevesi, H. javanicus, T. sibiricus. C. erythraeus, S. niger*), 2/11 among 50 and 100 (*N. nasua, M. coypus*) and 4/11 higher than 100 (*O. zibethicus, N. procyonoides, S. carolinensis, P. lotor*).

Table 3 - Number of articles, total observed pathogen species richness, and observed pathogen species richness per pathogen taxon

Host	N articles	Observed pathogen species richness	Bacteria	Virus	Helminths	Protozoa	Ecto-parasites
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
TOT	1055	993	191	92	427	90	188

The GLMM showed that the observed pathogen species richness was positively influenced by the number of articles and that this influence differs among pathogen taxa, with some classes (virus and bacteria) showing a higher effect than the others (Table 4, Figure 2A). On the other hand, the number of hosts sampled showed a different effect among parasite taxa, with no evident general positive effect (Figure 2B). With regard to the area of studies, the observed pathogen species richness in the area of origin resulted to be significantly higher than in the area of introduction (Figure 2C).

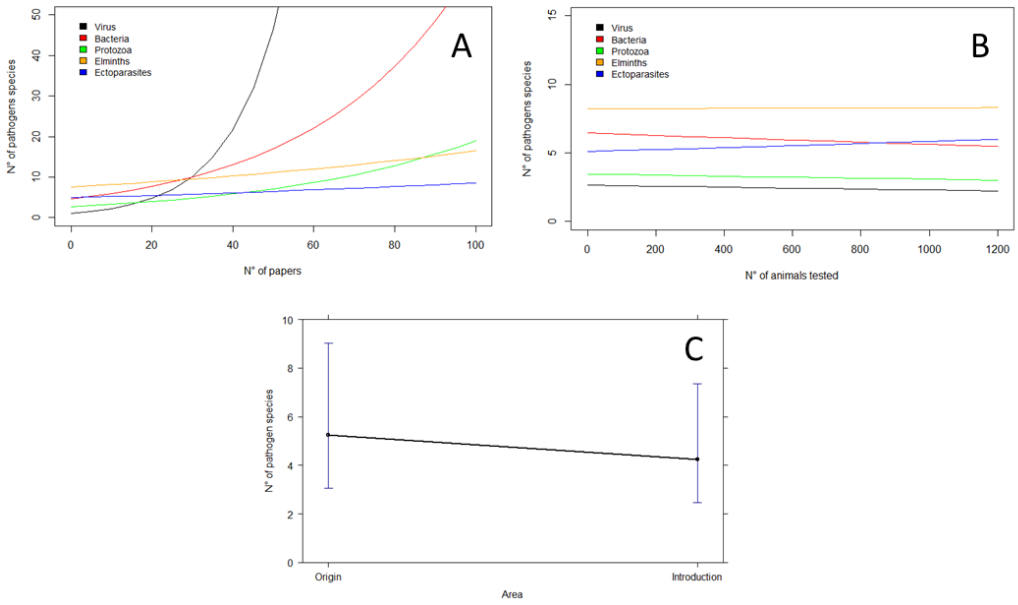

Figure 2 - (A) Effect of the n° of articles on the n° of pathogens species observed, per pathogen taxa (virus in black, bacteria in red, protozoa in green, helminths in yellow, and ecto-parasites in blue), and (B) effect of the n° of animals sampled on the n° of pathogens species observed, per pathogen taxa.
(C) Effect of the area on the n° of pathogen species observed

Table 4 – Results of Generalized Linear Mixed Model analyzing factors explaining the observed pathogen species richness

Variable	d.f.	χ^2	P value
Area	1	5.937	0.014
Pathogen taxon	4	36.869	<0.001
N° of articles	1	12.161	<0.001
N° of hosts sampled	1	0.101	0.750
Pathogen taxon: N° of articles	4	12.355	0.014
Pathogen taxon: N° of hosts sampled	4	13.528	0.008

The IAS species accumulation curves fitted with the number of articles as measure of the sampling effort showed the curve asymptotes (i.e. the estimated helminth species richness) varying from a minimum of 16 (*T. sibiricus*) to a maximum of 254 (*P. lotor*) (Figure 3 and Table 5). For two species, data were insufficient to compute the accumulation curves (*M. reevesi* and *H. javanicus*). The comparison of the observed helminth species richness with the estimated one revealed that the observed helminth species richness covers more than the 50% of the estimated helminth species

richness only for 3/11 IAS (*S. carolinensis, P. lotor, N. procyonoides*), while it covers less than 50% for 6/11 species (*M. coypus, O. zibethicus, T. sibiricus, N. nasua, S. niger, C. erythraeus*) (Table 5).

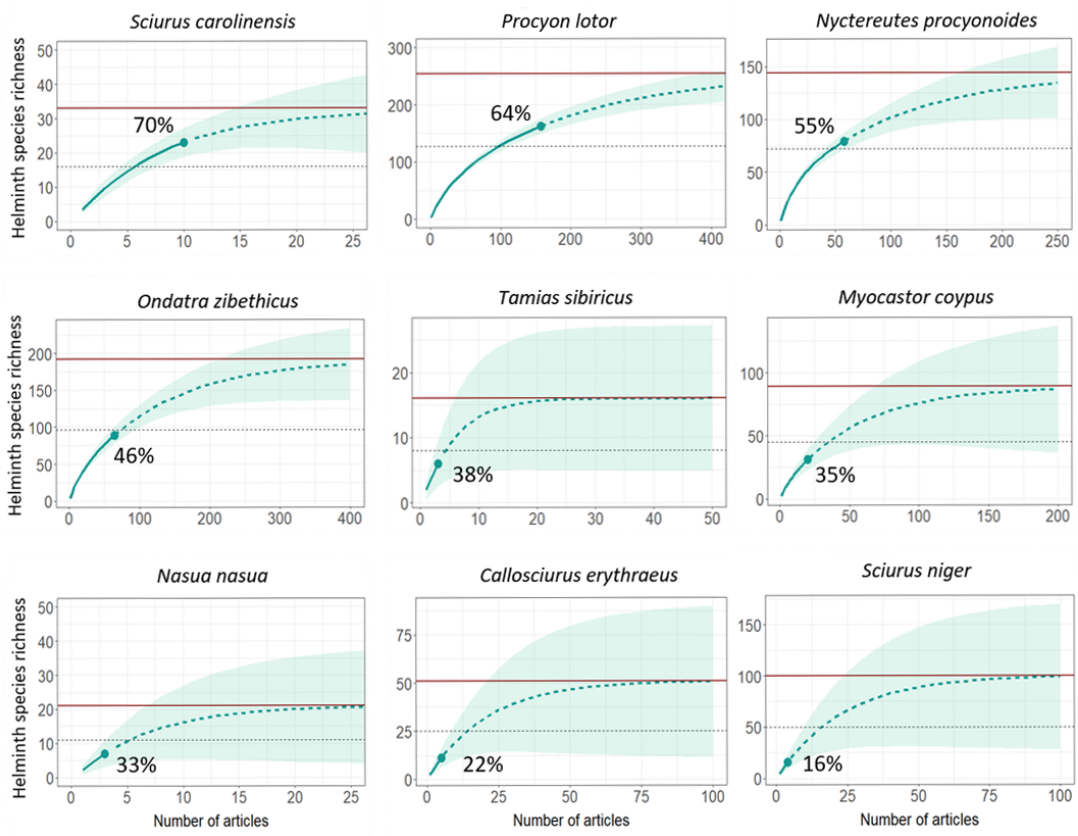

Figure 3 – Helminth species accumulation curves for each host species.
Red lines represents the curve asymptote, black dotted lines the 50% of the curve asymptote.

Table 5 - Observed helminth species richness, estimated helminth species richness with confidence intervals (CI), and coverage of the observed helminth species richness on the estimated one (%)

	Observed helminth species richness	Estimated helminth species richness	95% C.I. lower	95% C.I. upper	Observed helminth species richness on the estimated helminth species richness (%)
Sciurus carolinensis	23	33	26	57	**70**
Procyon lotor	162	254	213	326	**64**
Nyctereutes procyonoides	79	144	106	231	**55**
Ondatra zibethicus	89	192	138	305	**46**
Tamias sibiricus	6	16	8	52	**38**
Myocastor coypus	31	89	48	221	**35**
Nasua nasua	7	21	10	64	**33**
Callosciurus erythraeus	11	51	17	264	**22**
Sciurus niger	16	100	30	516	**16**

Muntjacus reevesi	3	NA	NA	NA	**NA**
Herpestes javanicus	0	NA	NA	NA	**NA**

Meta-analysis of pathogens of public and animal health significance

With regard to the sanitary relevance of the reported pathogens, the majority of the IAS analyzed showed to have more than 10 pathogen species of public and/or animal health significance (Table 6). Full list of pathogen species of public and animal health significance identified is available in **Supplementary material S4**. The lowest and highest number of pathogens of health significance identified per IAS were respectively 1 for *M. reevesi* and 38 for *P. lotor* (Table 6).

Table 6 - Number of pathogen species of public and animal health significance identified through the literature review (pathogens of the same genus with similar clinical outcome were grouped together)

IAS	Pathogens relevant to (n):			
	Public health	Livestock health	Wildlife health	**Public and animal health**
Procyon lotor	22	17	15	**38**
Nyctereutes procyonoides	10	8	9	**19**
Myocastor coypus	10	7	5	**16**
Sciurus carolinensis	12	5	5	**14**
Ondatra zibethicus	8	6	4	**13**
Nasua nasua	6	5	3	**11**
Sciurus niger	6	2	4	**7**
Herpestes javanicus	5	2	-	**5**
Tamias sibiricus	4	2	1	**4**
Callosciurus erythraeus	2	-	1	**2**
Muntiacus reevesi	-	1	1	**1**

Each host species, on average, was found to have only the 30% of its relevant pathogens with sufficient data to perform a meta-analysis of prevalence. In particular, it was not possible to estimate a pooled prevalence for any of the relevant pathogens identified for *C. erytraeus, H. javanicus* and *M. reevesi*. 3/11 IAS resulted to have less than the 40% of their relevant pathogens with data available for meta-analysis (*T. sibiricus* 25%, *S. niger* 29%, *N. nasua* 36%), 3/11 between the 40% and

50% (*M. coypus* 38%, *S. carolinensis* 43%, O. *zibethicus* 46%), and 2/11 higher than 50% (*N. procyonoides 53%* and *P. lotor 53%)*.

Pooled prevalence of the pathogens with data available for meta-analysis were obtained from an average number of studies varying from 3 (*T. sibiricus*) to 14 (*P. lotor*), with 4/11 IAS with data available for meta-analysis having on average a number of studies per pathogen lower than or equal to five (n=3: *T. sibiricus*, n=4: *S. niger, N. nasua, S. carolinensis*), and 4/11 IAS higher than five (n=6: *N. procyonoides*, n=7=*M. coypus* ; n=9: *O. zibethicus*, n=14: *P. lotor*).

Considering the meta-analysis outputs (Figure 4, **Supplementary Material S5**), the pathogens revealed large confidence intervals for the pooled prevalence estimates, with the 18% (10/55) of the pathogen species having pooled prevalence IC larger than 50%, the 29% (16/55) among 20% and 50%, the 30% (17/55) among 10% and 20%, and only the 23% (12/55) lower than 10. The subgroup meta-analyses per area of study (see **Supplementary Material S5**) showed that the 42% (17/41) of the pathogens have a difference of more than 10 percentage points among the pooled prevalence in the area of origin and introduction.

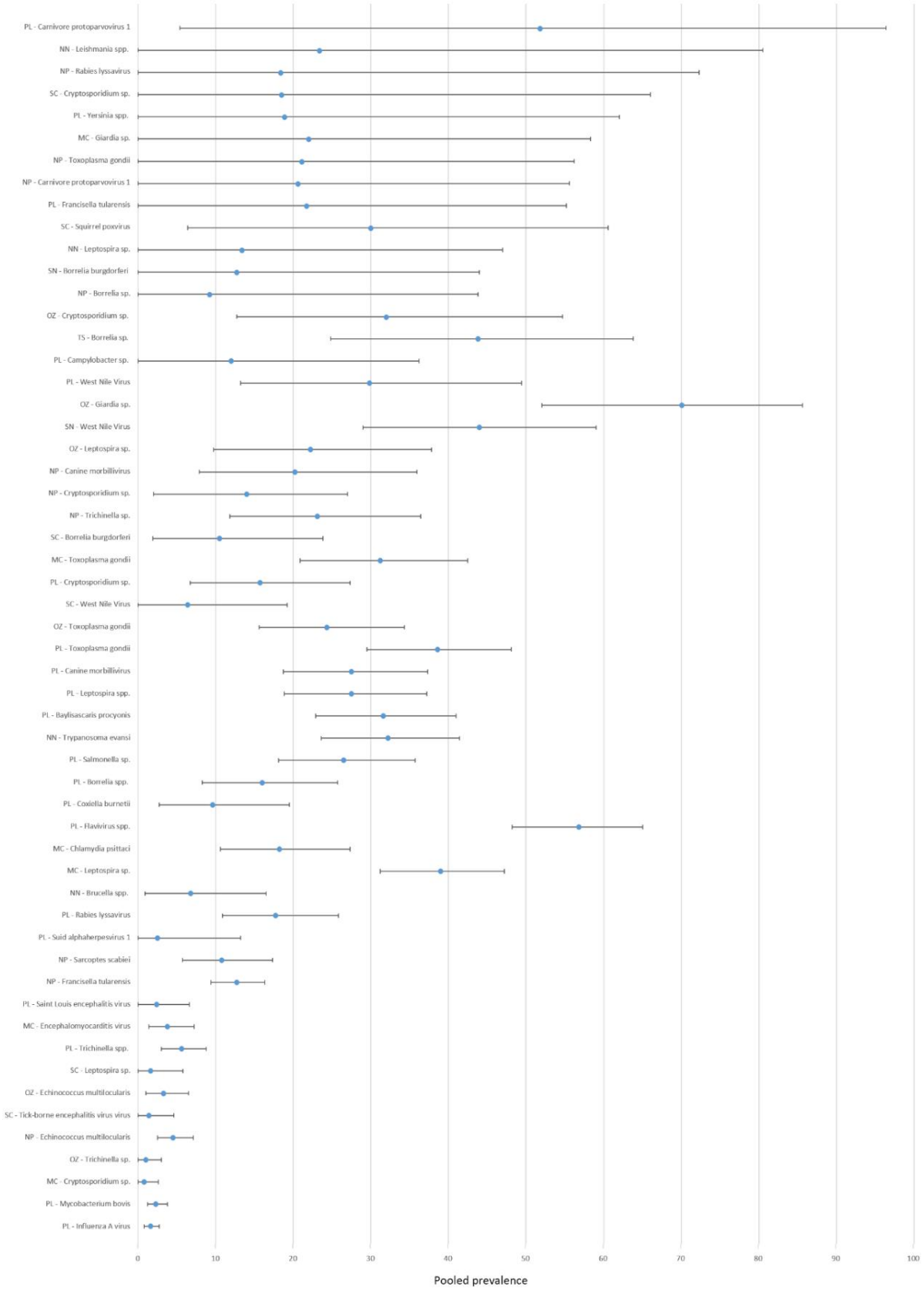

Figure 4 – Forest plot with the pooled prevalence of the pathogens of health significance identified in the host IAS analyzed. PL=Procyon lotor, *NN*=Nasua nasua, *NP*=Nyctereutes procyonoides, *SC*=Sciurus carolinensis, *MC*=Myocastor coypus, *SN*=Sciurus niger, *OZ*=Ondatra zibethicus, *TS*=Tamias sibiricus.

Discussion

In this study, we systematically reviewed the literature to explore the current knowledge on the pathogens harbored by mammal IAS. More specifically, through different statistical approaches, we (i) analyzed the main factors associated with research intensity and the observed pathogen species richness, (ii) estimated the true pathogen species richness, and (iii) evaluated the pooled prevalence of pathogens of public and animal health significance. Results highlighted (i) the existence of strong information gaps and heterogeneity in the way research on the pathogens of mammal IAS is carried out, (ii) the current underestimation of the amount of pathogens harbored by these species and (iii) the existence of high levels of uncertainty in the prevalence estimates of the pathogens of public and animal health significance.

The investigation of the main factors associated with research intensity, measured in term of both the number of articles and the number of sampled individuals, showed that, whilst the different pathogen taxa appear to be studied to the same extent, research intensity varies significantly among host species and areas (area of origin vs area of introduction). The existence of heterogeneous taxonomic research intensity has already been found to characterize the study of IAS biological and ecological impacts (Pyšek *et al.*, 2008; Hulme *et al.*, 2013), and such biases have also been found to characterize the study of the human health impacts of plant and animal IAS in Europe (Shindler *et al.*, 2015). However, focusing on the risk related to infectious agents, this may represent an important gap in our preparedness against zoonoses, as species belonging to taxa notoriously implicated in the epidemiology of zoonotic diseases (e.g., rodents) (Woolhouse, Haydon and Antia, 2005;

Jones *et al.*, 2008; McFarlane, Sleigh and McMichael, 2012; Han, Kramer and Drake, 2016) resulted to be scarcely investigated. With regard to the area of study, IAS pathogens were found to be more investigated in the host native areas, respect to the areas of introduction. While acknowledging that this may be due to the fact that the introduction of some species are relatively recent events, this highlights the actual poor understanding of the infectious disease dynamics of invasive species in their areas of release. It is indeed well-acknowledged that IAS, besides potentially introducing new pathogens, may also affect local infectious disease dynamics by acquiring, and potentially, amplifying, the endemic ones (Dunn, 2009; Chinchio *et al.*, 2020), and to the aim of assessing IAS disease risks, it is critical to have a knowledge of the pathogens that these species harbor in both their native and introduced areas.

The analysis of the best predictors of the observed pathogen species richness showed the number of articles to be a better predictor respect to the number of hosts sampled. This not obvious outcome, may be interpreted by the fact that our systematic review encompassed studies with very different purposes. As such, the number of pathogens does not necessarily increase in parallel with the number of hosts sampled, as very large samples may be analyzed with the aim to search for a single pathogen, whilst smaller samples with the aim to identify multiple pathogens species. This applies in particular to some taxa like viruses and bacteria, where pathogen investigations are usually more specific than those for macro-parasites, and may explain why the effect of the number of articles on the observed pathogen richness for these two taxa resulted much higher than that of other taxa.

The effect of the area of sampling as predictor of the observed pathogen species richness, which is higher in the area of origin, merits attention since the so-called "enemy release hypothesis" (Torchin *et al.*, 2003) states that the number of parasites in the area of introduction is usually lower than that in the area of origin. This hypothesis has relevant sanitary implications, as a better understanding of the factors driving the phenomenon would allow to identify the species most likely to successfully introduce and harbor pathogens of health significance. Our results highlight how it is essential to evaluate for uneven research intensity among areas when testing for this hypothesis, in order to prevent the outcome from being affected by the higher research intensity in the area of origin.

The estimation of the true pathogen species richness and its comparison with the observed one provided an estimation of the dimension of the disease risk that a species may represent, and allowed us to be more aware of the proportion of this risk we currently know. Estimating the unknown true pathogen species richness of a host species is a challenge, and to this aim we adapted here statistical techniques used in ecology to estimate biodiversity in vertebrates, plants and parasites (Dove and Cribb, 2006). Such methods are usually applied to data obtained from studies specifically designed to assess the presence of a selected taxon or group of pathogens in a species (see for example the application of Anthony *et al.* 2013 to estimate bats viral richness), and we acknowledge that the richness estimator may be influenced by its application on heterogeneous data belonging to studies with different aims. In particular, the estimator could be influenced by the presence of studies which focus only on specific pathogen species. Hence, the choice to apply this method on helminths, as usually parasitological analyses on helminths, unlike

those for virus and bacteria, are able to reveal a broad spectrum of species present in the organ investigated.

The comparison of the observed helminth species richness with the estimated one suggests that we are far from reaching a realistic knowledge of the parasite community characterizing these host species, even for the best known ones. With the exception of the grey squirrel, only two other host species were found to have an observed helminth species richness covering more than the half of the estimated true helminth species richness. However, as these host species were found to harbor a large number of helminth species, this still implies that they host hundreds of species of which we are not aware. As it is evident from the large confidence interval of the curves (Fig. 3), the remaining host species were far less studied, and in two cases, data were so scant that it was not even possible to compute the species accumulation curves. Although it could be argued that the lack of knowledge on IAS helminths may characterize wild species in general, the lower investigation of IAS in the areas of introduction respect to the area of origin suggests that wild species introduced in new areas (i.e., IAS) are actually less known.

While acknowledging the intrinsic limits of applying species accumulation curves on heterogeneous literature data, explorative analyses on the overall pathogen species richness computed including all the pathogen taxa showed, in general, similar patterns (see **Supplementary Material S6**). This may suggest that our conclusions could also extend to all the pathogen taxa, and not only to helminths.

Lastly, our investigation on the IAS pathogens of health significance showed that mammal IAS harbor several pathogens with relevant public health implications

(e.g., rabies, leptospirosis, Lyme borreliosis) and with recognized impacts on both the welfare and productivity of domestic animals and biodiversity conservation (e.g., bovine tuberculosis, avian influenza, canine distemper). However, at the same time, results revealed how our knowledge on the role of IAS in their epidemiological dynamics is often very limited, as studies evaluating the prevalence of these infections were mostly unavailable, and even in case they were, the pooled prevalence showed confidence intervals so high to raise serious questions on the extent to which inference may be made based on these data. Unfortunately, IAS, as other wild animals in general, are indeed often sampled opportunistically without a proper statistical planning (Guberti, Stancampiano and Ferrari, 2014), and we frequently observed studies with low sample sizes or heterogeneous sample structure. Additionally, the fact that many pathogens are characterized by a high variability in the pooled prevalence estimates among areas, highlights how the risk of an IAS introducing new pathogens in an area of release may vary greatly according to the IAS site of origin. Making inference based on restricted data which do not necessary reflect the epidemiological situations of other contexts, may thus lead to important over- or underestimation of disease risk.

Concluding, our results confirm how our current knowledge on mammal IAS infections is lacking. These results are even more relevant if we consider that the species here analyzed represent the 25% of the mammal IAS established in Europe (Genovesi *et al.*, 2009), and, being these species enlisted in the European Regulation, they should be among the well-known. Further work would be required to establish if the same knowledge gaps characterize other taxa of health relevance,

like birds. The available information, despite all its limitations, confirms that mammal IAS harbor a wide range of pathogens with possible health implications, supporting the need for ad-hoc disease risk assessments to identify priority IAS species on which to concentrate research and management efforts. Resources to investigate the health status of wildlife are indeed limited, and empirical research on IAS pathogens should be directed to the species representing the highest risks, for which statistically sound epidemiological investigations should be carried out. However, current knowledge gaps may importantly constrain our ability to perform disease risk assessments using as inputs literature data. To partially overcome this issue, it would be advisable to develop risk assessment methods which do not solely rely on literature data for informing the risk model, but instead on techniques of expert knowledge elicitation (EFSA, 2014). Meanwhile, more efforts are recommended in making the available information on IAS pathogens more accessible and systematized, implementing the existing IAS databases with information related to their pathogens.

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CHAPTER 4

Development of a tool to prioritize animal Invasive Alien Species based on their disease risk

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Manuscript

1. Introduction

Wildlife plays a key role in the circulation and emergence of infectious agents (Daszak, 2000; Jones *et al.*, 2008), and its correct management is essential to prevent the rise of diseases harming both public and animal health. Animal invasive alien species (IAS) are wild species introduced by humans in areas where they are not found naturally, and in which they spread uncontrollably harming local biodiversity and producing huge economic losses. Besides altering the natural dynamics that regulate local ecosystems, they represent as well a potential health threat (Crowl *et al.*, 2008; Conn and Conn, 2014; Hulme, 2014; Chinchio *et al.*, 2020). In particular, acting as pathogen hosts, IAS may introduce new micro-organisms and/or acquire and possibly amplify those endemic to the area of release, posing a potential threat to local human and animal populations (Chinchio *et al.*, 2020).

The increasing number of IAS worldwide, due to globalization, makes untargeted management actions towards them unfeasible, and the development of strategies aimed at their prioritization a necessity (McGeoch *et al.*, 2016). Several governments have enacted specific legislation and intervention plans, with the primary aim to prevent IAS introduction and spread and minimize their impacts. The European Union, for example, has recently issued a regulation setting up a list of species of Union concern (Reg. EU 1143/2014 and related implementing acts), to be updated continuously according to the output of transparent risk assessments aimed at estimating IAS risks towards biodiversity, economy and human health (European Union, 2014, 2016, 2017, 2019).

Risk assessment procedures allow indeed to estimate the likelihood and the magnitude of adverse impacts and represent a valuable instrument to guide and implement policies and actions. During the last years, more than 300 risk approaches have been developed in the field of invasion biology (Leung 2012), but the attention remained mainly focused on IAS environmental impacts (Essl *et al.*, 2011; Hulme, 2014; Srebaliene *et al.*, 2019). Even when impacts on human health are considered, they are evaluated as "general impacts on human health" (Srebaliene *et al.*, 2019), without a specific evaluation of the infectious agents that IAS may host and the dynamics that may occur with local populations, leading to the possible emergence of infectious diseases in both humans and native animals.

The need to increase the collaboration among invasive species biologists and health professionals with the aim to develop approaches to assess animal IAS infectious disease risk has been brought to attention (Conn and Conn, 2014; Hulme, 2014; Chinchio *et al.*, 2020), but currently, only a single procedure partially answered this need in the context of terrestrial animal IAS, allowing to assess the risk posed by a selected pathogen harboured by a plant or animal IAS (D'hondt *et al.*, 2015).

In this context, our aim is therefore to propose a risk assessment tool specifically designed to allow the prioritization of mammal IAS based on their infectious disease risk, here defined as the likelihood that these species, once established in a selected geographic area, act as hosts contributing to the transmission of relevant infectious agents to humans, livestock and/or native wildlife. These three targets allow to consider the impact of IAS on public health, the economy and welfare of production animals, and biodiversity conservation. As the lack and fragmentation of information

on pathogens hosted by IAS makes the existing databases and literature an insufficient input data source to inform risk assessments procedures (see Chapter 3 of the present volume), the tool functioning relies on expert opinion and on a qualitative methodology, less data-demanding and time-consuming than a quantitative one.

Such tool, applied to the invasive species more likely to be introduced and spread in a selected region and/or to those already introduced in the area, allows their prioritization from the human and/or animal health point of view. Its application to different geographical contexts may thus help implementing the existing local legislation, while at the same time, through the estimation of uncertainty, identifying the species and pathogens for which further empirical research is needed.

In this paper, we describe the tool and, for illustrative purpose, we apply it to assess the zoonotic disease risk of two IAS with reference to the Italian context: the already established American grey squirrel *Sciurus carolinensis* and the raccoon *Procyon lotor*.

2. Methods

2.1 Tool development

The tool consists in a qualitative disease risk assessment methodology applicable to any actual or potentially invasive mammal species in a defined area, in order to assess the risk that an established population of this species poses/would pose to humans, livestock and/or native wildlife (here referred to as the target populations), through the transmission of infectious agents (new or endemic to the area) that may adversely impact them. Tool users are therefore responsible to define their

specific risk question as regard to the IAS, the target population, and the region for which performing the risk assessment, as well as the origin of the infectious disease risk of interest, differentiating among the risk that the IAS pose to the target by transmitting new pathogens not endemic to the area (here defined "risk of introduction"), or pathogens endemic to the area (here defined "risk of amplification").

It has to be noted that this tool does not allow to evaluate the risk of the IAS to be introduced and becoming established in the selected area, but it is specifically intended to evaluate the disease risk associated with the IAS, assumed that it has succeeded in being introduced and establishing in the area. The tool is therefore intended to represent an instrument for local administrators and disease managers to identify which IAS (whether they are actually established in the area, or not) should be prioritized in terms of preventive/management actions.

The disease risk assessment methodology has been developed according to the OIE/IUCN guidelines for wildlife disease risk analysis (Jakob-Hoff *et al.*, 2014). In particular, here we deal with two risk analysis steps: a) hazard identification, where the pathogens to take into account in the risk assessment are defined, and the actual b) risk assessment, where the chain of events (i.e. the risk pathway) leading from the release of each hazard to the target infection are described and their likelihoods are estimated. The results of the foregoing steps are integrated for each pathogen according to the risk model, and the tool output consists in a list of pathogens with their risk to be transmitted from the IAS to the target population, expressed in qualitative terms.

The tool is expert-based, meaning that the input information needed to perform the risk assessment, like the likelihoods and uncertainty estimations of the risk

pathway events, have to be elicited from experts through an ad-hoc questionnaire (available in **Supplementary Material S1**), which has been structured following the risk assessment steps.

Further details on the risk assessment process, the qualitative risk model, and the questionnaire are given below.

2.1.1 Risk assessment

2.1.1.1 Hazard identification

Here we define as hazards the infectious agents able to produce an adverse effect in the target population (humans, livestock or native wildlife), to which the IAS of interest is susceptible.

Since a comprehensive checklist of the most relevant pathogens affecting mammal IAS is not available, we derived a list of relevant pathogens affecting each target population from international legislations and Health Organisations, and in particular we referred to the zoonotic pathogens listed in the "Commission Implementing Decision (EU) 2018/945 on communicable diseases to be covered by epidemiological surveillance" for human target, the OIE list of notifiable diseases and the European "Animal Health Law" Regulation (EU) 2016/429 pathogen list for livestock target, and the list of diseases affecting wild animals defined by the OIE Working Group on wildlife diseases for wildlife target (see **Supplementary Material S3 of Chapter 3** for the complete lists of pathogens).

Based on the target of interest, the experts are presented with one of these lists and identify the pathogens to which the IAS is susceptible, also considering, in the lack of data, the phylogenetic proximity with the species commonly affected by the

pathogen. The list of pathogens is non-restrictive, thus that if the IAS is known to harbour a pathogen relevant to the target not included in the list, for example an emerging pathogen, it is always possible to include it in the assessment.

Moreover, experts are asked to specify for each pathogen if it is endemic to the area under assessment, thus that the risk assessment will proceed only for pathogens relevant to the risk question, based on the infectious disease risk of interest ("risk of introduction" or "risk of amplification"). For livestock and wildlife target, experts are asked to specify if a target is present for each pathogen, thus that if the pathogen under assessment has no relevant domestic or wild hosts in the area, the risk assessment for that pathogen is stopped.

2.1.1.2 Risk pathways and risk factors

A risk pathway, i.e. the chain of steps representing the events that may lead a pathogen spreading from the IAS population to the target population, has been defined, for both non-vector borne (Fig. 1) and vector-borne pathogens (**Supplementary Material S2**), and the main factors influencing each event of the pathway according to the transmission route of the pathogen considered have been defined for each target population. The risk pathway includes a release and an exposure assessment. As the same pathogen may be preferentially transmitted through different routes according to the communities of hosts involved and the specific context (e.g. *Yersinia pestis* can be either transmitted through vectors or direct contact), the non-vector-borne and the vector-borne pathways are not mutually exclusive, and experts are asked to identify and refer to the most likely transmission route for each step of the pathway.

Both pathways and factors were validated through distinct informal discussions with four experts in wildlife epidemiology belonging to universities (Alma Mater Studiorium Università di Bologna) and national organisations (Istituto Zooprofilattico Sperimentale delle Venezie, Istituto Zooprofilattico Sperimentale della Lombardia e Dell'Emilia Romagna, Istituto Superiore per la Protezione e la Ricerca Ambientale), in which they provided their feedback on the general epidemiological mechanisms involved in the pathways and the key factors to consider. In particular, the information hardly available or unknown were identified and omitted from the assessment process. For example, due to the lack of information, the directionality in the transmission among vectors and host populations has not been considered, such that the likelihood of vectors transmitting the pathogen to a population and of the same population transmitting the pathogen to vectors was assumed to be equal.

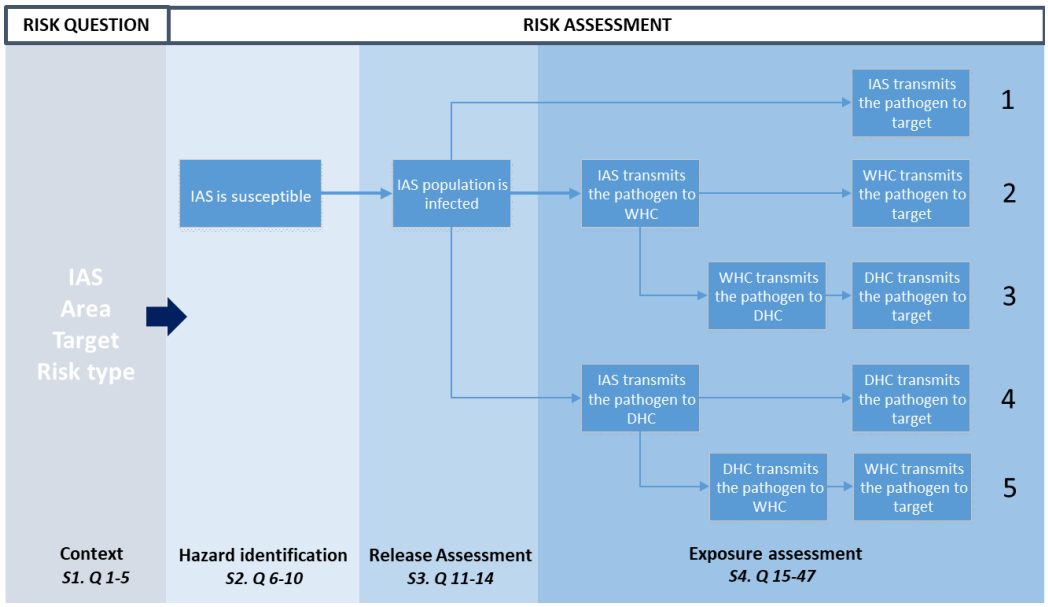

Figure 1 – Risk pathway for non-vector-borne pathogen, where target can be alternatively considered humans, livestock, and native wildlife. Five sub-pathways have been identified: (1) Transmission IAS-target, (2) Transmission IAS-WHC-target, (3) Transmission IAS-WHC-DHC-target, (4) Transmission IAS-DHC-target, and (5) Transmission IAS-DHC-WHC-target. In the lower part of the figure are indicated the different risk assessment stages along with the correspondent sections in the questionnaire reported in Supplementary Material 1.
IAS=Invasive Alien Species, WHC=Wild Host Community, DHC=Domestic Host Community.

Release assessment

The release assessment of the identified hazards evaluates their likelihood to infect an actual or potential IAS population, and thus of being potentially released from the IAS into the environment, where susceptible target species may be exposed. Here, experts assess the likelihood of the IAS established population to be infected with each hazard, considering the data available (in case the IAS under assessment is already established in the area of interest), or a series of factors (in case the IAS is currently absent from the area, or no data on the established population are available). These factors are: the most likely origin of the introduced individuals (affecting the likelihood of the founding population to be infected the pathogen), the pathogen cycle and virulence (affecting the likelihood of successful co-introduction of the pathogen with its host), the environmental conditions and the presence and density of the hosts/vectors needed to the pathogen in the area of release (affecting the successful establishment of the pathogen). If the pathogen is endemic to the area of introduction, additional factors to consider are represented by local pathogen circulation and the presence of species that may transmit the pathogen to the IAS, which affect the likelihood of the IAS of acquiring the pathogen locally.

Pathogens assessed to have a negligible likelihood of infecting the IAS population are not further investigated through the risk assessment process.

Exposure assessment

The exposure assessment evaluates the likelihoods of the events that may lead the target population to be exposed to each hazard. Here, we identified five sub-pathways (see Fig. 1), considering the local host populations, which may create new

multi-host pathogen dynamics with the invasive species (Jackson, 2002; Bohm *et al.*, 2007; Sepulveda *et al.*, 2014). The target may indeed be exposed directly to the infected IAS (or to vectors/environments/food infected by the IAS, according to the pathogen transmission route), or through the intermediation of local competent host species, i.e. local species able to transmit the pathogen. For the sake of simplification, local competent host species were considered as part of a competent host community (here defined as the community of species able to transmit the pathogen to other individuals), and in particular, a competent wild host community (WHC) and a competent domestic host community (DHC), comprehensive of both livestock and pets. This differentiation was introduced to take into account for the different factors influencing pathogen transmission when wild or domestic hosts are involved (Gortazar *et al.*, 2007; Lloyd-Smith, 2009).

The biotic and abiotic factors related to the IAS population, the local competent host communities and the area of release identified to influence the likelihoods of the events included in the exposure assessment are:

- IAS/WHC/DHC structure in terms of density and distribution in the area;
- IAS/WHC/DHC behavioural patterns (e.g. sociality, prey preferences, habitat preferences);
- IAS/WHC/DHC susceptibility to the pathogen and competence in its transmission (both intra- and inter- specific) and maintenance;
- Local vectors density, competence and host preferences;
- Local environmental characteristics (e.g. presence of wind, water, mechanical vectors);

- Local animal husbandry characteristics (e.g. farming type, access to pasture, farm biosecurity level);
- Human occupational or recreational activities (e.g. encroachment of human settlements into sylvatic areas, outdoor activities) (if target are humans);
- Local customs and practices (e.g. eating of raw animal products, sanitary conditions) (if target are humans or livestock).

The described baseline pathway is valid for all target populations. To not over-complicate the model, the likelihood of transmission from humans to livestock and/or wildlife has been considered as negligible and was not included in the pathway.

2.1.2 Risk model

2.1.2.1 Likelihood and uncertainty assessment

The event likelihoods are expressed in five qualitative categories: negligible, very low, low, medium and high, and the relative uncertainties are expressed in terms of high, medium or low (Table 1).

Uncertainties were taken into account with the acknowledgement that our current knowledge of IAS infectious diseases and related ecological dynamics is strongly limited. For this reason, respondents are told to evaluate uncertainty not only based on the literature available, but, in the lack of it, based on their personal evaluations.

Table 1- Likelihood and uncertainty levels definitions

LIKELIHOOD	DEFINITION
Negligible	Probability of event sufficiently low to be ignored or event only possible in exceptional circumstances
Very low	Probability of event is rare but cannot be excluded
Low	Occurrence of event is a possibility in some cases
Medium	Occurrence of event is a possibility
High	Occurrence of event is clearly a possibility
UNCERTAINTY	**DEFINITION**
Low	Solid and complete data available; strong evidence provided in multiple references; authors report similar conclusions; in case references are not available, experts answering the survey are very confident on the likelihood based on their personal evaluations
Medium	Some but no complete data available; evidence provided in small number of references; authors report conclusions that vary from one another; in case references are not available, experts answering the survey are quite confident on the likelihood based on their personal evaluations
High	Scarce or no data available; evidence is not provided in references but rather in unpublished reports, based on observations, or personal communication; authors report conclusions that vary considerably between them; in case references are not available, experts answering the survey are not confident on the likelihood based on their personal evaluations

2.1.2.2 Risk estimation

As the five sub-pathways are composed by conditional events, the likelihood estimates in each sub-pathway are combined through a matrix for dependent steps, where the resulting likelihood can never be higher than the lowest likelihood of the two events (Dufour *et al.*, 2011, Gale *et al.*, 2010) (Table 3). To combine the uncertainty of the different steps for each sub-pathway, we followed a precautionary approach such that the uncertainty estimate of a sub-pathway is equal to the maximum uncertainty estimate of the sub-pathway steps.

We therefore obtain five likelihoods and the related uncertainties, one for each sub-pathway, representing the likelihood of the target to be infected as a consequence of each possible interaction between the IAS and the local host communities. The overall risk and uncertainty estimates deriving from the combination of the five sub-pathways, are then obtained through the method described in Chapter 5 of the present volume.

Table 2- Matrix for dependent steps

	NEGLIGIBLE	***VERY LOW***	***LOW***	***MEDIUM***	***HIGH***
NEGLIGIBLE	*Negligible*	*Negligible*	*Negligible*	*Negligible*	*Negligible*
VERY LOW	*Negligible*	*Very low*	*Very low*	*Very low*	*Very low*
LOW	*Negligible*	*Very low*	*Low*	*Low*	*Low*
MEDIUM	*Negligible*	*Very low*	*Low*	*Medium*	*Medium*
HIGH	*Negligible*	*Very low*	*Low*	*Medium*	*High*

2.1.3 Data collection from experts

A questionnaire reflecting the events that constitute the risk pathway has been developed to elicit from experts the input data needed to perform the risk assessment, in particular those related to the hazards and the qualitative estimates of the likelihood and uncertainty related to each event.

At the beginning of the questionnaire, general guidelines are provided to the experts on the questionnaire structure and how to answer to questions, in particular to refer to the worst-case scenario when different scenarios are possible.

The questionnaire is composed of four sections, the first one (**Supplementary Material S1**, section S1) allows to define the specific risk question of interest, i.e., the species, the area, the target and the origin of the infectious disease risk, while the subsequent three sections (**Supplementary Material S1**, sections S2, S3 and S4) follow the risk assessment stages (i.e. hazard identification, release assessment and exposure assessment). Questions can require different type of answers, but only questions evaluating the likelihood of the steps presented in the risk pathway are fed into the risk model. Other questions may serve to define which answers will be shown, if a selected sub-pathway needs to be stopped, or to better guide respondents in their evaluations (see **Supplementary Material S1** for further details). To

standardize the answers as much as possible, questions evaluating the events likelihoods explicitly state the factors to take into account when providing the estimation, and experts should provide a brief rationale for each answer, referring to these factors.

As answering requires a throughout knowledge of the biology of the IAS subject of the risk assessment, the main wild and domestic species present in the selected area and the infectious disease dynamics of the main local zoonotic, livestock and wildlife infectious diseases, the questionnaire should be administered to a multidisciplinary working group of experts including IAS biologists, wildlife ecologists and veterinary epidemiologists working in the area under assessment.

2.2 Application test

For illustrative purpose, the tool was applied to assess the zoonotic infectious disease risk of two IAS with reference to the Italian context: the already established American grey squirrel *Sciurus carolinensis* and the raccoon *Procyon lotor*. The questionnaire was administered to a small working group of two experts in wildlife epidemiology and IAS ecology. Information collected through the questionnaires were evaluated according to the risk model to produce the final risk estimates with the relative uncertainty levels.

3. Results of the application test

The application of the tool identified as hazards 28 and 29 pathogens to which the raccoon and the grey squirrel may be susceptible. Of these, 7 at risk of introduction and 21 at risk of amplification for the raccoon, and 7 at risk of introduction and 22 at risk of amplification for the grey squirrel.

In particular, the raccoon resulted to represent a high risk for 4 pathogens, a medium risk for 3 pathogens, a low risk for 1 pathogens, a very low risk for 1 pathogen and a negligible risk for the remaining 19 pathogens; 1 pathogen resulted to have high uncertainty, 15 medium and 12 low (Figure 2). The grey squirrel resulted to represent a medium risk for 3 pathogens, a low risk for 1 pathogen, and a negligible risk for 25 pathogens; four pathogens resulted to have high uncertainty, 13 medium and 12 low (Figure 2).

All the pathogens at risk of introduction resulted to have a negligible risk, for both the host species; uncertainty levels for pathogens at risk of introduction resulted to be low for all but one pathogen for the raccoon, and for all but two pathogens for the grey squirrel (Figure 3).

The risk estimates, and, when applicable, the five sub-pathways estimates related to each pathogen, are reported in **Supplementary Material S3**.

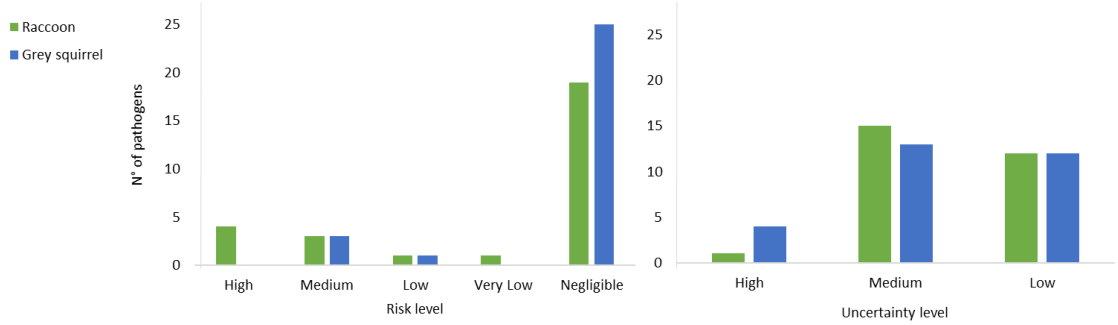

Figure 2 - Risk assessment results (risk and uncertainty estimates) for raccoon (green bars) and grey squirrel (blue bars)

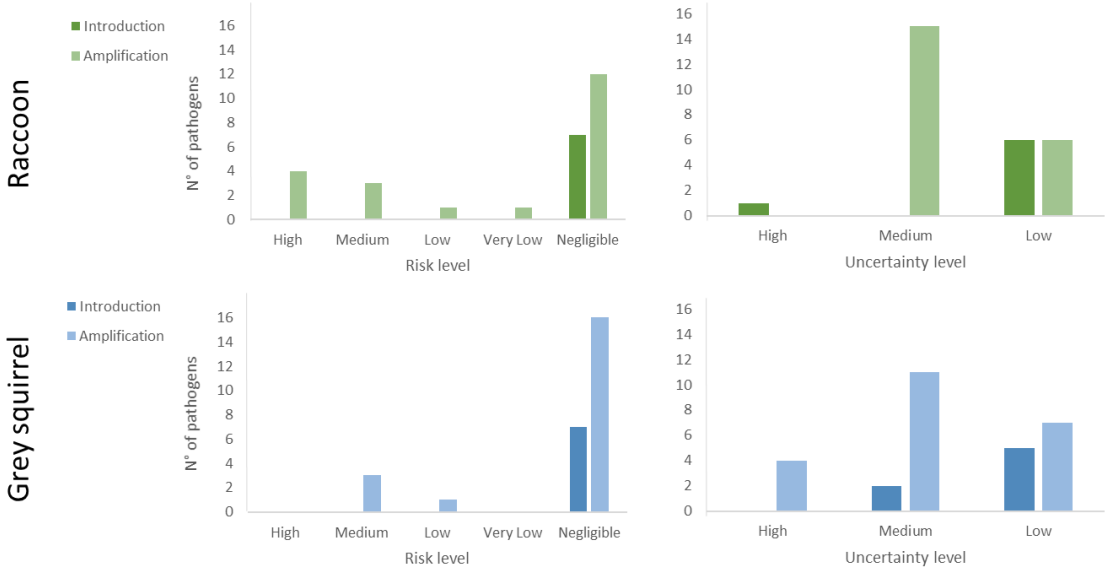

Figure 3 – Raccoon and grey squirrel risk assessment results (risk and uncertainty estimates) per origin of infectious disease risk (pathogens at risk of introduction and pathogens at risk of amplification)

4. Discussion

In this paper we presented a risk assessment tool to estimate the increase in local infectious disease risk consequent to the establishment of a mammal IAS harboring pathogens of health significance. This tool, for each IAS, provides as output a list of the pathogens of animal and human health significance that the host species could transmit to a population of interest, accompanied by their level of risk and uncertainty, thus that inference on the priority host species and pathogens can be drawn.

A key feature of the tool is its flexibility. A recognized issue in risk assessment methods applied in the context of invasive species is indeed their often high specificity in terms of species and/or geographical contexts, which hinders the pursuing of a coordinated approach among countries (McGeoch *et al.*, 2016). The tool was therefore designed to allow for its application to different areas, mammal species, targets (human, livestock and native wildlife), and origin of infectious disease risk (risk of introducing new pathogens and risk of amplifying local ones). For instance, the risk of acquiring and transmitting local pathogens is often overlooked in favor of the risk of introducing new pathogens (Roy *et al.*, 2017; Ogden *et al.*, 2019). When applying the tool to wild animal target, it has to be acknowledged that there are important limitations in the prediction of risks derived from the introduction of new pathogens, as the impact of many alien pathogens on biodiversity remain unknown until they are introduced (Roy *et al.*, 2017). Nevertheless, the tool still allows to identify risks related to the introduction of pathogen species with known biodiversity impacts, e.g., Canine Distemper Virus (Gutierrez and Ruiz-Saenz, 2016).

Besides being highly flexible, one of the main added values of our tool respect to other existing methods (see e.g. D'hondt *et al.*, 2015), lies in the fact that it does not necessarily require to define a priori which pathogens to evaluate. Instead, it requires a complete screening of the pathogens of health significance that the IAS may host, stimulating experts reasoning even for species characterized by a considerable lack of data (see Chapter 3 of the present volume), while always permitting the inclusion of additional known hazards, if needed; this gives also the possibility to risk managers to gain further insights in the main critical pathogens identified. Another advantage of our approach is that, as the risk pathway has been explicitly defined,

the tool allows for a high resolution of the mechanisms under assessment, making possible for risk managers not only to identify the most critical pathogens, but also the most critical sub-pathways and, through the rationale provided by experts, the most critical factors (e.g. a low level of farming biosecurity, or particular local risky customs), such that targeted actions can be put in place.

Prioritizing hazards in the absence of data is a challenge, nevertheless it is an urgent priority from a preventive point of view, and qualitative risk assessments provide valuable alternatives to help with decision-making processes for an efficient allocation of resources. The rationale underlying the development of this tool is the willingness to provide a procedure more transparent as possible, while always keeping in mind the consistent lack of data in the field of IAS-borne pathogens (Hulme, 2014; Chinchio *et al.*, 2020, and see Chapter 3 of the present volume). The existing knowledge gaps, as well as the broad range of pathogens considered, imposed a series of choices and simplifications in the model.

First, it precluded us the development of a quantitative methodology, and directed the choice to rely on expert opinion for the input data. Our envisioned process includes the formation of a working group of experts with experience in the area under assessment and belonging to several disciplines, including infectious disease epidemiologists and wildlife ecologists. Discussion should be open and stimulated through the entire questionnaire, as the assessment of each step involves aspects requiring both the expertise. It is highly advisable to follow standardized procedures for eliciting knowledge from experts, as the one described in EFSA (2014).

Second, the risk pathway was simplified as much as possible in terms of both the steps and the factors to consider (for example, we combined together the events related to the "exposure" and the "infection" of an host with a pathogen in a single "transmission" step), and the experts are not asked to separately assess each potential risk factor affecting a pathway step, but are left free to evaluate which factors to consider among those provided based on the available information, while always providing a rationale for their choices and reflecting the lack of data in the uncertainty estimations.

Lastly, it is important to discern which aspects were not covered here, as these may represent opportunities for future work.

First, we focused uniquely on the possibility of the IAS increasing local disease risk through its role as pathogen host. IAS, however, can influence infectious disease risk through other indirect mechanisms, like predation and competition with local hosts, possible changes in the quality of the habitat that may affect local hosts' behavior and fitness, or by altering population dynamics of disease vectors (Chinchio *et al.*, 2020). Moreover, IAS may even reduce infectious disease risk in some cases (see "dilution effect", Keesing *et al.*, 2006). As these ecological mechanisms are very difficult to predict, being highly context-dependent and requiring a throughout knowledge of local wild populations (Ostfeld and Keesing, 2012), we decided to not include them in our analysis. Hopefully, these additional pathways may be considered in the future, if further insights in the driving factors of the indirect increase of disease risk will be gained through ecological studies.

Moreover, here we assessed the likelihood that the target population can be infected with the pathogen, but we did not performed an assessment of the different

consequences that this may represent for the dynamics of the disease in the area, for example, if the species presence could provide a route for an endemic pathogen to a previously unexposed target, lead to epidemic events of the disease in the target or to the endemisation of the pathogen in the area. These aspect may be worth to be investigated for pathogens resulted at highest risk of exposure and included in the model in the future.

Considering the increasing translocation of species, vectors and pathogens among countries also favored by climate change, it could also be interesting to evaluate the potential impacts that the IAS might have in the area if a disease that is currently absent should be introduced. For example, in our application test on the raccoon in Italy, its disease risk for rabies resulted as negligible, considering the fact that rabies is known to be currently absent in the country. Assessing the potential risk of the host species for pathogens currently absent in the area would instead allow to evaluate the risk of raccoon if rabies should be introduced in the future, for example, through the movements of foxes from neighboring eastern countries, where the disease is endemic.

Another aspect that was not considered here is represented by the magnitude of the pathogens in terms of social and economic costs. All the listed pathogens are relevant to human and animal health in general, but it is to risk managers to interpret the tool output based on their relative importance in the local context. If impact assessment on these pathogens are available in the area under assessment, it could be interesting to combine their evaluations with the output provided by our tool.

Finally, while we created this tool for mammal IAS, we believe that it could be adjusted to take into account for the specificities of other taxa as well.

In conclusion, our tool provides a flexible and transparent way to assess and compare IAS disease risk, allowing for the consideration of health aspects when directing preventive and management actions towards mammal IAS.

Considering the current lack of information in the field of IAS pathogens, we believe that the application of this tool to several IAS in different areas may be beneficial not only to risk managers, but also for the future development of the field, as the results obtained would allow to identify the most critical pathways and mechanisms, as well as the most critical IAS and pathogen species, gaining insights on the biological drivers of IAS disease risk, with possible future applications in predictive models.

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CHAPTER 5

How to “sum” words? A proposed method to combine estimates in qualitative risk assessments

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Manuscript

1. Introduction

Qualitative risk assessments are widely used in the context of food safety, animal and public health to assess the risk of human exposure to foodborne pathogens (WHO and FAO, 2008), animal infection or hazards being introduced into a region (World organisation for animal health, 2008; Jakob-Hoff *et al.*, 2014; Peeler, Reese and Thrush, 2015). While quantitative risk assessments provide numerical estimates of the risk (e.g. probability of introduction, exposure or infection) and rely on strict mathematical principles, outputs of qualitative risk assessments are by definition non-numerical descriptors (i.e. likelihoods), such as "*High"*, "*Medium"*, "*Low"* or "*Negligible"* (World organisation for animal health, 2008).

Being defined by the International Organisation for Animal Health (OIE) as a "reasoned, logical and referenced discussion" of the available scientific evidence (World organisation for animal health, 2008), qualitative risk assessments are inevitably more subjective than quantitative risk assessments. Nonetheless, despite some well recognized methodological limitations (Cox, 2008), qualitative frameworks often represent the most useful approach in scarce-data settings or when a rapid response is needed to tackle new or urgent threats, like emerging infectious diseases (Palmer, Brown and Morgan, 2005; Morgan *et al.*, 2009) and outbreaks (ECDC, 2019); for example, a qualitative risk assessment to estimate the risk of food or food contact materials as a transmission route for SARS-CoV-2 has been conducted recently by the Food Standard Agency in UK (Oakenfull *et al.*, 2020).

In both qualitative and quantitative risk assessments, the structure of the model is informed by a risk pathway outlining the necessary events for the hazardous

event to occur (e.g. introduction, exposure or infection). However, while in quantitative risk assessment the risk pathway is subsequently translated in mathematical terms by using probability distributions, and the output(s) are obtained by means of computer simulations, the same mathematical operations cannot be performed with likelihoods (Kelly *et al.*, 2018).

Likelihoods are usually combined in pairs through a matrix defining the resulting estimate, presenting the process by the logic implication: "*If likelihood for event A is x and likelihood for event B is y, the resulting likelihood is z*" (see eg. Gale *et al.*, 2010; Wieland *et al.*, 2011). The subjectivity of qualitative models is therefore inherent not only in the choice and definition of the ordinal scale describing the likelihoods, but also in the way these estimates are combined. It should be however noted that, behind the qualitative terms used to define each likelihood, there is invariantly the perception of a numerical range. For this reason, although subjective, it is usually adopted the rationale that qualitative approaches to combine likelihoods should conceptually approximate quantitative probabilistic approaches.

As, along a risk pathway, all the events have to occur for the negative event of interest to happen, the combination of likelihoods (i.e. Event A ∩ Event B ∩ Event C etc...) to obtain the final risk estimate inspires to the multiplication rule of probabilities, and the following principles are usually adopted (Dufour *et al.*, 2011):

(i) Combining a 'null' probability with any other level of probability results in a 'null' probability of occurrence.

(ii) The smallest end result is 'nearly null', except when combining a 'null' probability with any other probability.

(iii) Combining two probabilities gives a result no higher than the lowest of the two levels.

However, a risk might arise from multiple pathways; for example, pathogen X may be introduced into a farm or a country through several routes, and in such cases the final risk estimate should result from a cumulative combination of the individual likelihoods of all the relevant pathways.

This methodological aspect has rarely been tackled in the literature. To our best knowledge, three approaches are mainly used to obtain the overall cumulative likelihood:

(i) Defined through subjective estimation on a case-by-case basis, without the use of formal methodologies (see e.g. Hartley, 2010);

(ii) Defined as the highest estimate amongst those that need to be combined (see e.g. Oakenfull *et al.*, 2020);

(iii) Derived through the use of a matrix (see e.g., Wieland *et al.*, 2011).

All these methods present in our opinion some drawbacks:

(i) Subjective estimations jeopardize the reproducibility and the robustness of the assessment;

(ii) The use of the worst estimate is a conservative approach which may prove useful in some situations, but it makes difficult to discriminate among several outputs where there is a need for a higher level of resolution and differentiation (for example, the combination of 3 "*Low"*, 10 "*Low"*, or 1 "*Low"* with 3 "*Very Low"*, would all produce the same output: "Low");

(iii) The use of matrices allows to evaluate only the combination of two input variables at a time. If used to combine additively more than two inputs, it is possible

that the final output varies according to the order in which the pair of inputs are selected, which is clearly undesirable, as a sum should not vary based on the order of the addends. For example, using the matrix applied in Wieland *et al.* (2011) to combine the likelihoods of non-dependent steps where an increase of risk is possible (see Table 1), the combination of three estimates "*Negligible"*, "*Negligible"* and "*High"*, would produce an overall result equal to "*Low",* if estimates are combined in the order "*High"-"Negligible"-"Negligible"* (as "*High*" + "*Negligible*" = "*Moderate*"; and "*Moderate*" + "*Negligible*" = "*Low*") or "*Negligible"-"High"-"Negligible"* (as "*Negligible"* + "*High"* = "*Moderate"*; "*Moderate"* + "*Negligible"* ="*Low"*), but equal to "*Moderate"* if they are combined in the order "*Negligible"-"Negligible"-"High"* (as "*Negligible"* + "*Negligible"* = "*Negligible"*; "*Negligible"* + "*High"*=" *Moderate"*).

Table 1 - Matrix to combine likelihoods of non-dependent steps where an increase of risk is possible from Wieland et al. (2011)

	Negligible	**Low**	**Moderate**	**High**
Negligible	Negligible	Low	Low	Moderate
Low	Low	Low	Moderate	Moderate
Moderate	Low	Moderate	Moderate	High
High	Moderate	Moderate	High	High

The ideal method to obtain a cumulative likelihood estimate from the combination of likelihoods should be applicable to any number of pathways and, in case the method is used for prioritization purposes, allow for a sufficient resolution (Duijm, 2015).

Following these considerations, the objective of this study is to contribute to the methodological development of qualitative risk assessments frameworks by presenting a systematic method for deriving a cumulative estimate from the combination of multiple likelihoods (or, more in general, of multiple qualitative estimates). The method is intended to improve the transparency and robustness of qualitative models.

2. Methods

The method proposed in this study is inspired to the pairwise (or cascade) summation, a method typically used for deriving the sum of *n* floating numbers in numerical analysis (Higham, 1993; Isupov, 2020), aimed at reducing the accumulated rounding error as compared to the traditional sum in sequence.

2.1 Pairwise summation of likelihoods

Given a number n of likelihoods L to be combined cumulatively and resulting in $L_{1,n}^{C}$, this method develops as follow:

(i) The likelihood estimates to be combined additively should be ordered in increasing order so that L_1 is the likelihood with the lowest categorical estimate and L_n the highest; this prevent to obtain results dependent from the order in which the estimates are combined.

(ii) Starting from L_1, likelihoods are combined additively by pairs so that: $L_{1,2}^{C} = L_1 + L_2$ where $L_{1,2}^{C}$ is the cumulative likelihood derived from the combination of L_1 and L_2.

(iii) $L_{1,2}^C$ is then combined additively with $L_{3,4}^C$ resulting in $L_{1,4}^C$, and the cascade process continues until $L_{1,n}^C$is computed.

A graphical representation of the method is outlined in figure 1.

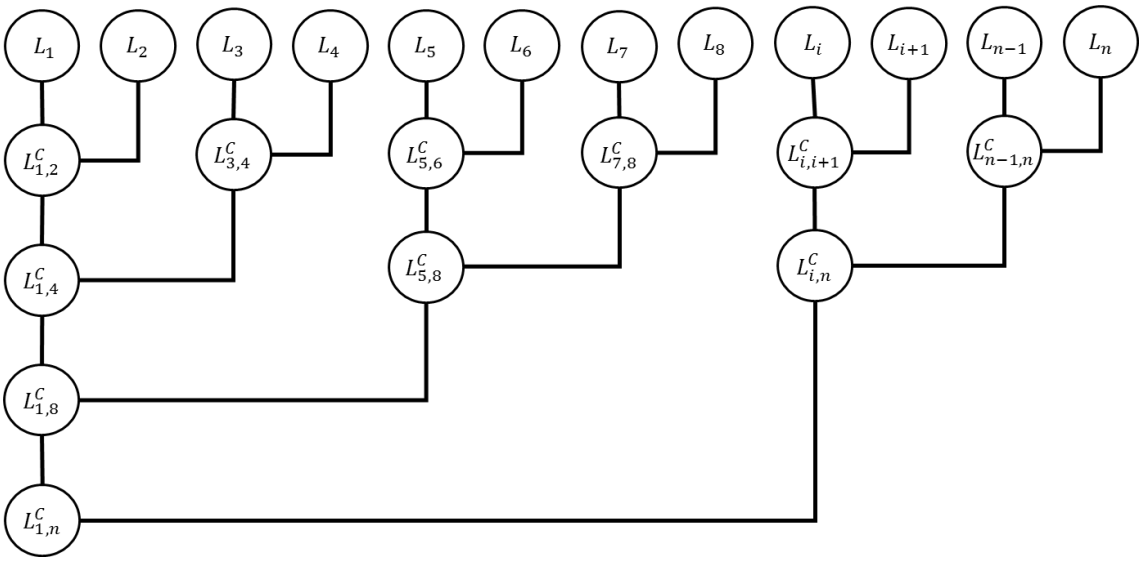

Figure 1. Computation of the final cumulative likelihood using the pairwise summation method

Along the process:

- If L_i and L_{i+1} are equal, $L_{i,i+1}^C$ is equal to the next categorical estimate (i.e., if L_i=*Low* and L_{i+1}=*Low,* $L_{i,i+1}^C$= *Medium*), except when L_i and L_{i+1} are both equal to the maximum categorical estimate possible, and when they are are both *Negligible*, case in which $L_{i,i+1}^C$ should remain *Negligible.*
- If L_i and L_{i+1} are different, $L_{i,i+1}^C$ is equal to the higher of the two likelihoods (i.e. if L_i=*Low* and L_{i+1}= *Medium,* $L_{i,i+1}^C$= *Medium*).

These assumptions are justified by the necessity of adhering as much as possible to the mathematical principles while dealing with likelihood terms, as explained below. Whatever is the choice of the likelihood scale (i.e. a four levels likelihood scale: *Negligible, Low, Moderate* and *High* or a five levels likelihood scale: *Negligible, Very Low, Low, Moderate, High* and *Very High*), conceptually, the likelihood scale covers the whole range of probability 0-100%. In purely qualitative risk assessments, the numerical ranges behind the likelihoods are not explicitly defined, and when two equal likelihoods (e.g. "*Low"* and "*Low"*) are combined additively, the only logical assumption that can be made is that the resulting cumulative likelihood should be "higher" than *"Low"*.

To prevent a rapid escalation of the likelihoods to the higher level, we propose to not apply the same principle when combining different likelihoods; for example, assuming that if L_i and L_{i+1} are different, $L^C_{i,i+1}$ is equal to the next categorical estimate, the additive combination of: "*Very Low*", "*Very Low*", "*Very Low*" and "*Low*" would result in "*High*". This would be unrealistic considering that a likelihood such as "*Very Low*" is generally used to describe a very rare event (EFSA, 2006). The instruction of posing each i cumulative likelihood $(L^C_{i,i+1})$ equal to the higher of the two likelihoods when L_i and L_{i+1} are different would indeed lead to the more reasonable estimate "*Low*".

2.2 Uncertainty

The qualitative estimates along the risk pathway(s) are often presented together with another qualitative term describing the magnitude of the uncertainty that is associated to each likelihood. As for the likelihoods, categorical ordinal scales are

also used to define the different levels of uncertainty (see for example uncertainty table in EFSA, 2006; Crotta, Ferrari and Guitian, 2016). As the uncertainty represents the lack of knowledge, when the likelihoods are combined "multiplicatively" the level of uncertainty that is normally (and conservatively) associated to the final estimate corresponds to the higher level of uncertainty encountered along the risk pathway. It is our opinion that the same should apply when likelihoods are combined additively. The cumulative likelihood should embed all the uncertainties of the risk pathways it derives and as such, a high uncertainty in one of them should be enough to have a high uncertainty associated to the cumulative likelihood as a consequence.

2.3 Illustrative example

For purpose of illustration of the pairwise summation method, the likelihood estimates provided by Hartley *et al.* (2013) are used. In that study, the authors presented a qualitative assessment of the risk for several deer species in Great Britain (i.e. roe, red deer, fallow deer, sika, muntjac and Chinese water deer) to be exposed to selected exotic infectious. The results obtained by Hartley *et al.* (2013) combining the estimates for the release assessment with those of the exposure assessment for red deer are summarized in Table 1 (in ascending order).

Table 1 - Likelihood estimate for the introduction of selected exotic infectious disease in red deer from Hartley et al. (2013)

Disease	Likelihood	Uncertainty
Enzootic Bovine Leukosis	*Negligible*	*Low*
Epizootic Haemorrhagic Disease	*Negligible*	*Low*
Warble Fly	*Negligible*	*Low*
Contagious agalactia	*Very Low*	*Medium*
Foot and Mouth Disease	*Very Low*	*Low*
Brucellosis	*Low*	*Low*
Vescicular Stomatitis	*Low*	*Medium*
Chronic Wasting Disease	*Low*	*Low*
Bluetongue	*Medium*	*Low*

Hence, applying the pairwise summation method it is possible to derive the cumulative estimate describing the overall likelihood of occurrence of at least one of the listed exotic infection in red deer in GB. This was done as described in section 2.1.

3. Illustrative example results

Results of the method applied to the likelihood estimates outlined in Table 1 are presented as worked diagram in Figure 2.

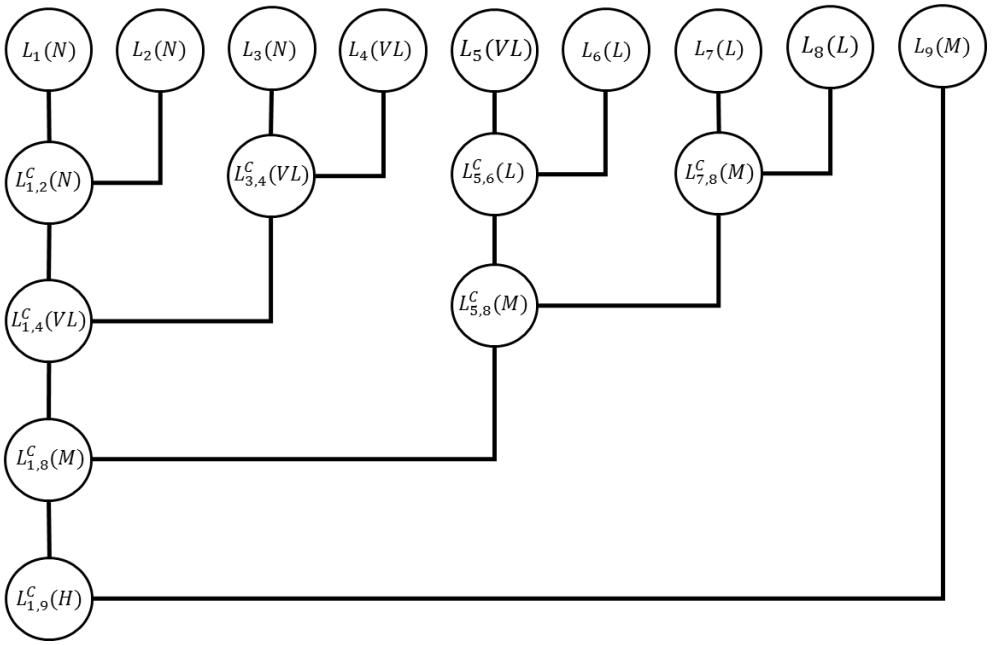

Figure 2 - Pairwise summation method applied to the case study, the likelihoods already presented in ascending order in Table 1 together with the resulting i cumulative likelihood ($\boldsymbol{L^C_{i,i+1}}$) are reported in brackets. N=Negligible, VL=Very Low, L=Low, M=Medium, H=High

The presence of at least one of the selected exotic infectious diseases in red deer in GB is estimated to be "*High*". The uncertainty, derived as the higher of the individual uncertainty estimates (see section 2.3), is estimated to be "*Medium*".

4. Discussion

In this study, in order to remedy the lack of consistency in the methods used to estimate the likelihood of an unwanted event happening through multiple pathways, we propose a standardized method to combine additively two or more qualitative estimates in a cumulative one. The method is inspired to the pairwise summation, a technique used in numerical analysis to sum a sequence of finite-precision floating-point numbers.

Due to the intrinsic nature of the risk concept, which comprehend components that are not absolute, it is not possible to define a real "value" to which refer in order to identify a "gold standard". This is even more true for qualitative models, due to their non-numeric nature. Whilst it is therefore not possible to define a method that is better than the others in every context, the method proposed here may prove useful when there is the need to combine more than two estimates and to obtain results with a high degree of resolution. Moreover, the fact that the method is standardized makes it repeatable, thus allowing for comparisons.

Despite acknowledging the major limitation of qualitative models, the method provide meaningful cumulative estimates in relation to the likelihood scale that is chosen. For example, considering a likelihood scale of five levels ("*Negligible*", "*Very Low*", "*Low*", "*Medium*" and "*High*"), eight "*Very Low*" risk pathways needs to be combined to lead to a "*High*" cumulative risk. This allows for a reasonable level of resolution and can be particularly useful if cumulative likelihoods of different entities needs to be compared for prioritization purposes (e.g. to evaluate the overall risk of introduction of at least one exotic disease into a country by different animal species).

The number of equal estimates that have to be combined to obtain the highest likelihood scale level depends from the number of the qualitative levels of the likelihood scale. Thus, in case a high number of estimates need to be combined and there is the necessity of an higher resolution in the likelihoods levels, it could be useful to define a higher number of qualitative levels. In this way, more events will be needed to reach the higher likelihood level .

The method also considers the uncertainty that is associated to the likelihoods combined additively. In this case, the uncertainties are not combined but the higher uncertainty encountered amongst the risk pathways is kept associated to the final cumulative likelihood. This is because uncertainties should not be combined in first instance; otherwise, even a "*Very Low*" uncertainty in all pathways would eventually result to a "*High*" uncertainty associated to the cumulative likelihood; which does not make sense. However, the value of keeping the uncertainty (in addition of providing the actual level of uncertainty for the estimate) is that the relative impact of the "lack of knowledge" for the single pathways can be explored by means of a qualitative sensitivity analysis (Crotta *et al.*, 2021).

Despite the inherent subjectivity, qualitative risk assessments remain widely applied either to support decision-making in scarce data settings or as a preliminary step to identify the risk pathways that merit more accurate quantitative investigations, and a key challenge in the field remains to improve the consistency of the underlying methods. The application of the method proposed here contributes to reduce qualitative models' subjectivity, moving from the application of diverse ad-hoc solutions to a standardized method, and thus increasing the transparency and reproducibility of qualitative risk assessments.

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CHAPTER 6

Conclusions and Perspectives

The aim of the present thesis was to frame IAS from a sanitary perspective, and to develop a methodology to assess their disease risk towards human and animal health. In particular, I focused my attention on the characterization of the hazards that mammal IAS species may represent to human and animal health, and to the development of a disease risk assessment methodology specific for the context of biological invasions (see Chapter 1).

First, in Chapter 2, I explored the existing ecological and biological literature on biological invasions to identify the mechanisms by which IAS may alter infectious disease dynamics in their area of release. IAS resulted to potentially affect disease risk by two main type of mechanisms: by acting as hosts of infectious agents (i.e. directly), thus possibly leading to the introduction of new pathogens, and/or the amplification of endemic ones, or by altering the abundance and/or contact rates among local host species, parasite infective stages and vectors through competitive and trophic interactions with native species or the modification of local habitats (i.e. indirectly). This literature review highlighted how IAS may have important health implications, which should be better acknowledged by people working in the human and animal health field, and how the mechanisms underlying the sanitary outcome of a biological invasion, and in particular indirect ones, are extremely complex, being the product of multiple factors. Acknowledging these important limitations of our comprehension of the topic, I addressed the issue of IAS disease risk by focusing specifically on IAS possible role as infectious agents' host. Being systematized information on IAS pathogens unavailable, the second Chapter of this thesis was devoted to systematically reviewing the literature to extract information on the infectious

agents harbored by the main mammal IAS. Data were then analyzed through different approaches, including multivariate regression analyses of the main factors associated to research intensity and to the observed species pathogen richness, the estimation of the true species pathogen richness, and meta-analyses of prevalence of the main pathogens of human and animal health significance. As it was expected, the results revealed important knowledge gaps. They allowed, however, to better characterize our knowledge, or, we could say, our lack of knowledge on the topic. Research resulted to be skewed towards a few species, with others being almost completely ignored. Uneven research intensity is known to characterize invasion literature (Pyšek *et al.*, 2008; Schindler *et al.*, 2015). However, in the epidemiological context this bias is even more dangerous, as all the species object of this study belong to taxa of recognized relevance in the epidemiology of zoonotic diseases, like rodents and carnivores. The existing bias is therefore not justified from the health point of view, and could lead us to underestimate potential risks. More worryingly, our knowledge on the pathogenic pool of these species resulted limited also for the most studied species, suggesting that many potential hazards remain still unknown. What remains certain, is that the review confirmed that mammal IAS harbor pathogens of human and animal health significance, like rabies, Lyme borreliosis, avian influence, echinococcosis and bovine tuberculosis, and the need to frame IAS from a health perspective is thus justified. However, a few pathogens had sufficient data available to perform a meta-analyses of prevalence, and even when it was possible, these estimates were characterized by high uncertainty levels, preventing us from drawing a clear picture of the role that IAS may have in the circulation of these path-

ogens. This finding highlights the necessity of conducting statistically robust analyses to better comprehend the epidemiological role of the high-risk species in the transmission of relevant infections. Presenting a method by which these high-risk species could be identified is the topic of Chapter 4, where I propose a qualitative disease risk assessment tool specifically aimed to assess IAS disease risk towards humans, domestic animal populations, or wildlife populations. The tool allows to obtain, for mammal IAS, a list of the pathogens of animal and human health significance that it could transmit to a population of interest (directly or through the communities of local hosts), each with the related level of risk and uncertainty. Respect to other existing methods, this tool not only is very flexible but, through the identification of all the steps possibly leading the IAS to transmit the pathogen to the target, allows for a high resolution of the mechanisms under assessment, making possible, if needed, to identify the most critical points. Finally, the necessity to combine multiple likelihood estimates deriving from several pathways in an overall risk estimate led me to tackle a methodological aspects of qualitative risk assessment methodologies in Chapter 5, where I proposed a standardized method to apply in such cases, to reduce the subjectivity that relies in the way multiple estimates are combined. While qualitative methodologies remain inevitably more subjective that quantitative ones, the identification of standard frameworks to apply to selected contexts, allows for an increased reproducibility and comparison of qualitative methods outputs.

With this thesis, I wanted to provide ground for the inclusion of biological invasions in the forecasts of future emerging diseases, covering a possible gap in our preparedness towards new health threats.

Until now, the possible health implications of biological invasions have been largely neglected, taking second place to the assessment of their environmental impacts, both in the research field than in policy-making. Nevertheless, here I highlighted how the role of IAS in disease dynamics might be currently underestimated and the urgent necessity to identify the species at highest priority to direct preventive actions, despite the scarcity of data. When an invasive species and/or an infectious agents begin to spread in an area, it is often too late to put in place solutions, and the costs may be enormous for both society and the economy. In epidemiology, as well as in invasion biology, prevention has to be a principle of choice and action, and should not be treated as an option (Saracci, 2020).

Expert opinion offers a partial solution to the current scarcity of data, but needs to be collaborative. For this reason it is fundamental that the risk assessment tool here presented is informed by multi-disciplinary groups of experts. While experts in IAS epidemiology may be lacking, experts in wildlife epidemiology, infectious disease dynamics and invasion biology are not, and their collaboration can allow to gain meaningful insights despite the scarcity of empirical data. A better definition of the process for eliciting opinions from experts, using recognized techniques to reduce biases (EFSA, 2014), may represent a future development for this work.

Moreover, the future application of this tool to define the disease risks of several IAS in different areas may be extremely beneficial not only to risk managers, allowing them to direct actions and surveillance plans, but also to research itself, as the

results obtained in different areas could be compared to identify the most critical pathways and mechanisms, as well as the most critical IAS and pathogen species, gaining insights on the biological drivers of IAS disease risk, with possible future applications in predictive models.

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Supplementary Materials

Chapter 3

Supplementary Material S1

Research string used to retrieve data about coypu (*M. coypus*) infectious agents adapted for each literature database; for other host species the string was modified by changing the common and scientific names of the IAS.

DATABASE	RESEARCH STRING
PubMed	(infectious[Title/Abstract] OR infection*[Title/Abstract] OR parasite*[Title/Abstract] OR parasitic[Title/Abstract] OR bacterial[Title/Abstract] OR bacteria[Title/Abstract] OR bacterium[Title/Abstract] OR viral[Title/Abstract] OR virus[Title/Abstract] OR viruses[Title/Abstract] OR protozoa*[Title/Abstract] OR zoonosis [Title/Abstract] OR zoonoses [Title/Abstract] OR zoonotic [Title/Abstract] OR "Bacterial Infections" [Mesh] OR "Virus diseases"[Mesh] OR "Parasitic Diseases"[Mesh] OR "Mycoses"[Mesh] OR "Parasitic Diseases, Animal"[Mesh] OR "Parasites"[Mesh] OR "Bacteria"[Mesh] OR "Viruses"[Mesh] OR "Zoonoses"[Mesh] OR "Infection"[Mesh] OR prevalence [Title/Abstract] OR occurrence [Title/Abstract] OR detection [Title/Abstract] OR identification [Title/Abstract] OR isolation [Title/Abstract] OR characterization [Title/Abstract] OR investigation [Title/Abstract]) AND (*Myocastor coypus* OR coypu* OR nutria OR nutrias)
CAB Abstract & Global Health	TS= ((infectious OR infection* OR *parasite* OR parasitic OR bacterial OR bacteria OR *bacterium OR viral OR *virus OR viruses OR protozoa* OR zoonosis OR zoonoses OR zoonotic OR prevalence OR occurrence OR detection OR identification OR isolation OR characterization OR investigation) AND (*Myocastor coypus* OR coypu* OR nutria OR nutrias))
Scopus	TITLE-ABS-KEY (infectious OR infection* OR *parasite* OR bacterial OR fungal OR viral OR parasitic OR bacteria OR *bacterium OR *virus OR viruses OR protozoa* OR zoonosis OR zoonoses OR zoonotic OR prevalence OR occurrence OR detection OR identification OR isolation OR characterization OR investigation) AND TITLE-ABS-KEY (*Myocastor coypus* OR coypu* OR nutria OR nutrias) AND NOT INDEX (medline)
Web Of Science Core Collection	TS= ((infectious OR infection* OR *parasite* OR parasitic OR bacterial OR bacteria OR *bacterium OR viral OR *virus OR viruses OR protozoa* OR zoonosis OR zoonoses OR zoonotic OR prevalence OR occurrence OR detection OR identification OR isolation OR characterization OR investigation) AND (*Myocastor coypus* OR coypu* OR nutria OR nutrias))

Supplementary Material S2

List of the articles extracted through the systematic review of literature and included in the analyses.

Available online at https://unimibox.unimi.it/index.php/s/pyZmK5Kmgaat8At

Supplementary Material S3

List of potential hazards for human target

Commission Implementing Decision (EU) 2018/945 of 22 June 2018 on the communicable diseases and related special health issues to be covered by epidemiological surveillance

Bacillus anthracis
Borrelia burgdorferi s.l.
Chikungunya virus disease
Clostridium botulinum
Corynebacterium diphtheriae, Corynebacterium ulcerans, Corynebacterium pseudotuberculosis
Coxiella burnetii
Creutzfeldt-Jakob disease
Cryptosporidium spp.
Dengue virus
Echinococcus multilocularis, E. granolosus
Giardia lamblia (syn intestinalis, duodenalis)
Hepatitis B
Human pathogenic Brucella spp.
Human pathogenic Campylobacter spp.
Influenza A/H5
Leptospira interrogans or any other pathogenic Leptospira
Listeria monocytogenes
Mycobacterium tubercolosis complex
Plasmodium spp.
Rabies virus
Salmonella enteritis
Salmonella typhi, S. paratyphi
Severe acute respiratory syndrome (SARS)
Shiga toxin/verocytotoxin-producing E. coli infection (STEC/VTEC)
Shigella spp.
Tick-borne viral encephalitis
Trichinella
Vibrio cholerae
Viral haemorrhagic fevers (VHF)
West Nile virus
Yellow fever virus
Yersinia enterocolitica/Yersinia pseudotuberculosis
Yersinia pestis
Zika virus

List of potential hazards for livestock target

OIE-Listed diseases (https://www.oie.int/animal-health-in-the-world/oie-listed-diseases-2020/)

African horse sickness virus
African swine fever virus
Anaplasma marginale,
Aujeszky's disease virus
Avian infectious bronchitis virus
Avian infectious laryngotracheitis (ILT) herpesvirus
Avian influenza viruses
Avian metapneumovirus
Babesia bigemina
Babesia caballi and theileria equi
Bacillus anthracis
Bluetongue virus
Bovine leukaemia virus (BLV)
Bovine viral diarrhea virus
Brucella abortus, brucella melitensis and brucella suis
BSE
Burkholderia mallei
Camelpox virus
Campylobacter fetus sbsp venerealis
Caprine arthritis encephalitis virus
Chlamydophila abortus(enzootic abortion of ewes, ovine chlamydiosis)
Classical swine fever virus
Crimean congo haemorrhagic fever virus
Duck hepatitis virus type 2 (DHV-2) and duck hepatitis virus type 3
Eastern equine encephalitis virus

Echinococcus granulosus
Echinococcus multilocularis
Ehrlichia ruminantium
Epizootic hemorrhagic disease virus (EHDV).
Equid herpesvirus-1 (EHV-1)
Equine arteritis virus
Equine infectious anaemia
Equine influenza virus
Francisella tularensis
Foot and mouth disease virus
Infectious bovine rhinotracheitis virus/infectious pustular vulvovaginitis virus
Infectious bursal disease (Gumboro disease)
Influenza a viruses of high pathogenicity in birds other than poultry including wild birds
Leishmania sp.
Lumpy skin disease virus
Maedi-visna virus
Mycobacterium bovis
Mycoplasma agalactiae bacteria in sheep and goats, M. capricolum capricolum, M. mycoides LC and M. putrefaciens in goats
Mycoplasma capricolum subsp. Capripneumoniae
Mycoplasma gallisepticum
Mycoplasma mycoides subsp. mycoides SC (contagious bovine pleuropneumonia)
Mycoplasma synoviae
Myxoma virus
Nairobi sheep disease orthonairovirus
New world screwworm (Cochliomyia hominivorax)
Newcastle disease virus
Nipah virus encephalitis
Old world screwworm (Chrysomya bezziana)
Ovine epididymitis (Brucella ovis)
Paratuberculosis
Pasteurella multocida
Peste des petits ruminants virus
Porcine reproductive and respiratory syndrome virus
Q fever
Rabbit haemorrhagic disease virus
Rabies virus
Rift valley fever virus
Rinderpest virus
Salmonella gallinarum
Salmonella pullorum
Salmonellosis (S. abortusovis)
Scrapie
Sheep pox and goat pox
Surra (Trypanosoma evansi)
Taenia solium (porcine cysticercosis)
Taylorella equigenitalis
Japanese encephalitis serocomplex of flaviviruses: alfuy (ALF); koutango (KOU); kokobera (KOK); kunjin (KUN); murray valley encephalitis (MVE); JE; stratford (STR); usutu (USU); and west nile (WN) st. Louis encephalitis (SLE)
Theileria
Transmissible gastroenteritis coronavirus (TGEV)
Trichinella spp.
Trichomonas gallinae
Trypanosoma congolense, t. vivax, and to a lesser extent t. brucei brucei. T. uniforme and T. simiae
Trypanosoma equiperdum
Venezuelan equine encephalitis virus
West Nile fever
Western equine encephalitis virus

Animal Health Law listed diseases

Regulation (EU) 2016/429 of the European Parliament and of the Council of 9 March 2016 on transmissible animal diseases and amending and repealing certain acts in the area of animal health ('Animal Health Law')

Japanese encephalitis virus
African horse sickness virus
African swine fever virus
Aujeszky's disease virus
Bacillus anthracis
Bluetongue virus

Bovine herpesvirus 1
Bovine leukaemia virus
Bovine viral diarrhea virus
Brucella abortus
Brucella melitensis
Brucella ovis
Brucella suis
Burkholderia mallei
Campylobacter fetus venerealis
Chlamydia psittaci
Classical swine fever virus
Coxiella burnetii
Eastern equine encephalitis virus
Ebola virus
Echinococcus multilocularis
Epizootic haemorrhagic disease virus
Equine arteritis virus
Equine Infectious Anaemia Virus
Foot and mouth disease virus
Goat pox virus
Highly pathogenic avian influenza virus
Low pathogenic avian influenza virus
Lumpy skin disease virus
Mycobacterium bovis
Mycobacterium caprae
Mycobacterium paratuboercolosis
Mycobacterium tubercolosis
Mycoplasma capricolum
Mycoplasma gallisepticum
Mycoplasma meleagridis
Mycoplasma mycoides
Newcastle disease virus
Peste des petits ruminants virus
Porcine reproductive and respiratory syndrome virus
Rabies virus
Rift Valley fever virus
Rinderpest virus
Salmonella arizonae
Salmonella gallinarum
Salmonella pullorum
Sheep pox virus
Taylorella equigenitalis
Trichomonas gallinae
Trypanosoma equiperdum
Trypanosoma evansi
Venezuelan equine encephalomyelitis virus
West Nile virus
Western equine encephalitis virus

List of potential hazards for wildlife target

Non OIE-listed diseases affecting wild animals
(https://www.oie.int/wahis_2/public/wahidwild.php/Diseaseinformation/popup/diseaselist)

Agent causing chronic wasting disease (CWD)
Alcelaphine herpesvirus 1 or Ovine herpesvirus 2
Avian Paramyxoviruses (other than those listed by the OIE)
Babesia spp. (new or unusual occurrences)
Baylisascaris procyonis
Borrelia spp.
Calicivirus in marine mammals
Circoviruses
Elephant endotheliotropic herpesviruses (EEHV)
Encephalomyocarditis virus (EMCV) – Cardiovirus A
Equine influenza (wild equidae)
European brown hare syndrome virus
Fasciola gigantica
Fascioloides magna

Feline leukaemia virus (FELV)
Filoviruses
Flavivirus (causing louping ill)
Flavivirus (causing tick borne encephalitis)
Flavivirus (causing yellow fever)
Hantaviruses
Henipaviruses (Hendra viruses)
Henipaviruses (Nipah viruses)
Histomonas spp.
Immunodeficiency viruses (Feline, Simian)
Leptospira interogans ssp.
Listeria monocytogenes
Low path. Avian influ. Viruses (all subtypes)
Morbillivirus (canids and felids)
Morbillivirus (marine mammals)
Morbillivirus in non-human primates
Newcastle disease virus (wild birds)
Parvoviruses
Pasteurella spp.
Plasmodium spp.
Pox viruses (other than those listed by the OIE)
Pseudogymnoascus destructans in bats (White-nose syndrome)
Psoroptes spp.
Salmonella enterica (all serovars)
Sarcoptes scabiei
Theileria spp. (new or unusual occurrences)
Toxoplasma gondii
Yersinia enterocolitica
Yersinia pestis
Yersinia pseudotuberculosis

Supplementary Material S4

List of pathogens of public and animal health significance identified for each host species.

HOST	Pathogen
Callosciurus erythraeus	*Cryptosporidium sp.*
	Leptospira interrogans
Herpestes javanicus	*Campylobacter sp.*
	Flavivirus Japanese encephalitis virus
	Leptospira sp.
	Lyssavirus Rabies lyssavirus
	Salmonella sp.
Muntiacus reevesi	*Theileria sp.*
Myocastor coypus	*Bacillus anthracis*
	Cardiovirus Cardiovirus A (EMCV)
	Chlamydia psittaci
	Clostridium botulinum
	Coxiella burnetii
	Cryptosporidium parvum
	Cryptosporidium sp.
	Echinococcus granolosus
	Echinococcus multilocularis
	Giardia sp.
	Leptospira interrogans
	Leptospira sp.
	Pasteurella multocida
	Salmonella sp.
	Toxoplasma gondii
	Yersinia pseudotuberculosis
	Brucella abortus

Nasua nasua	*Brucella canis*
	Brucella sp.
	Cryptosporidium sp.
	Flavivirus Ilheus virus
	Giardia sp.
	Leishmania infantum
	Leishmania shawi
	Leishmania sp.
	Leptospira sp.
	Mycobacterium bovis
	Orthopoxvirus sp.
	Theileria sp.
	Toxoplasma gondii
	Trypanosoma evansi
Nyctereutes procyonoides	*Amdoparvovirus sp.*
	Baylisascaris procyonis
	Betacoronavirus Severe acute respiratory syndrome-related coronavirus
	Borrelia sp.
	Cryptosporidium canis
	Cryptosporidium parvum
	Cryptosporidium sp.
	Echinococcus multilocularis
	Flavivirus Japanese encephalitis virus
	Francisella tularensis
	Giardia intestinalis
	Giardia sp.
	Influenzavirus A Influenza A virus
	Listeria monocytogenes
	Lyssavirus Rabies lyssavirus
	Morbillivirus Canine morbillivirus
	Parvovirus sp.
	Protoparvovirus Carnivore protoparvovirus 1
	Sarcoptes scabiei
	Toxoplasma gondii
	Trichinella sp.
	Trichinella spiralis
	Varicellovirus Suid alphaherpesvirus 1
Ondatra zibethicus	*Campylobacter sp.*
	Chlamydia psittaci
	Cryptosporidium parvum
	Cryptosporidium sp.
	Echinococcus multilocularis
	Flavivirus Omsk hemorrhagic fever virus
	Francisella tularensis
	Giardia intestinalis
	Giardia sp.
	Influenzavirus A Influenza A virus
	Leptospira interrogans
	Leptospira sp.
	Lyssavirus Rabies lyssavirus
	Orthohantavirus sp.
	Toxoplasma gondii
	Trichinella sp.
Procyon lotor	*Alphacoronavirus Alphacoronavirus 1 (TGEV)*
	Alphavirus Eastern equine encephalitis virus
	Alphavirus Venezuelan equine encephalitis virus
	Amdoparvovirus Carnivore amdoparvovirus 1
	Bacillus anthracis
	Baylisascaris procyonis

	Borrelia afzelii
	Borrelia burgdorferi
	Borrelia garinii
	Borrelia lonestari
	Borrelia sp.
	Borrelia turicatae
	Brucella abortus
	Brucella canis
	Brucella sp.
	Campylobacter jejuni
	Campylobacter sp.
	Clostridium botulinum
	Coxiella burnetii
	Cryptosporidium parvum
	Cryptosporidium sp.
	Flavivirus Japanese encephalitis virus
	Flavivirus Powassan virus
	Flavivirus Saint Louis encephalitis virus
	Flavivirus sp.
	Flavivirus West Nile Virus
	Francisella tularensis
	Giardia sp.
	Influenzavirus A Influenza A virus
	Leptospira borgpetersenii
	Leptospira interrogans
	Leptospira kirchneri
	Leptospira sp.
	Listeria monocytogenes
	Lyssavirus Rabies lyssavirus
	Morbillivirus Canine morbillivirus
	Mycobacterium avium
	Mycobacterium bovis
	Mycobacterium tuberculosis variant microti
	Orthopoxvirus Raccoonpox virus
	Orthopoxvirus sp.
	Parvovirus sp.
	Protoparvovirus Carnivore protoparvovirus 1
	Protoparvovirus sp.
	Salmonella enterica
	Salmonella sp.
	Sarcoptes scabiei
	Toxoplasma gondii
	Transmissible spongiform encephalopathies prions
	Trichinella murrelli
	Trichinella pseudospiralis
	Trichinella sp.
	Trichinella sp. T9
	Trichinella spiralis
	Varicellovirus Suid alphaherpesvirus 1
	Yersinia enterocolitica
	Yersinia pestis
	Yersinia pseudotubercolosis
	Yersinia sp.
Sciurus carolinensis	*Alphavirus Eastern equine encephalitis virus*
	Borrelia afzelii
	Borrelia burgdorferi
	Borrelia burgdorferi ss.
	Borrelia garinii
	Borrelia valaisiana

	Cryptosporidium sp.
	Flavivirus Saint Louis encephalitis virus
	Flavivirus sp.
	Flavivirus Tick-borne encephalitis virus
	Flavivirus West Nile Virus
	Francisella tularensis
	Giardia sp.
	Leptospira kirschneri serovar Grippotyphosa
	Mycobacterium bovis
	Salmonella enterica
	Sciuripoxvirus Squirrelpox virus
	Toxoplasma gondii
	Usutu virus
Sciurus niger	*Borrelia burgdorferi*
	Cryptosporidium sp.
	Flavivirus West Nile Virus
	Leptospira interrogans
	Lyssavirus Rabies lyssavirus
	Toxoplasma gondii
	Yersinia pestis
Tamias sibiricus	*Borrelia afzelii*
	Borrelia burgdorferi
	Borrelia burgdorferi ss.
	Borrelia garinii
	Borrelia sp.
	Coxiella burnetii
	Cryptosporidium parvum
	Francisella tularensis

Supplementary Material S5

List of the meta-analyzed pathogens. For each pathogen the total pooled prevalence (or the pooled prevalence when only one study was available) and the subgroup pool prevalence per area are reported.

AO=Area of Origin; AI=Area of Introduction; CI=Confidence Intervals

Host species	Pathogen species	IAS area	N° studies	Pooled prevalence/ prevalence	CI 95%
Myocastor coypus	*Chlamydia psittaci*	All	2	18.2	10.6-27.3
		AO	1	21	15.3-27.4
		AI	1	10.7	1.5-25.4
	Cryptosporidium spp.	All	4	0.8	0-2.6
		AO	1	3.8	0.8-8.3
		AI	3	0	
	Cardiovirus Cardiovirus A (EMCV virus)	All	3	3.8	1.4-7.2
		AO	1	3.4	1.1-6.7
		AI	2	3.5	0-11.6
	Giardia spp.	All	6	22	0-58.3
		AO	1	1.9	0-5.5
		AI	5	29.7	0-79.1
	Leptospira spp.	All	20	39	31.2-47.2
		AO	1	38.1	31-45.4
		AI	19	39.1	30.7-47.9
	Toxoplasma gondii	All	6	31.2	20.9-42.5
		AO	1	27.8	21.4-34.7
		AI	5	31.8	18.2-46.7
Sciurus carolinensis	*Borrelia burgdorferi spp. (incl. B. afzelii, garini, valaisiana e B. burgdorferi ss)*	All	4	10.5	1.9-23.8
		AO	1	40	11.5-72.3
		AI	3	6.6	0.9-16.8
	Cryptosporidium spp.	All	2	18.5	0-66
		AO	1	40.4	32-49
		AI	1	3.7	12-73
	Leptospira spp. (incl. L. kirshneri)	All	3	1.6	0-5.8
		AO	1	5	0-20.3
		AI	2	0	
	Squirrel poxvirus	AI	8	30	6.4-60.2
	Flavivirus Tick-borne encephalitis virus virus	AI	2	1.4	0-4.6
	Flavivirus West Nile Virus	All	8	6.4	0-19.2
		AO	4	14.9	0-46.2
		AI	4	1.5	0-4.8
Sciurus niger	*Borrelia burgdorferi*	AI	3	12.7	0-44
	West Nile Virus	All	4	44	29-59
		AO	3	42	19-67
		AI	1	48.6	40.3-56.9
Tamias sibiricus	*Borrelia spp. (incl. B. burgdorferi)*	AI	3	43.8	24.8-63.8
Ondatra zibethicus	*Cryptosporidium spp. (incl. C. parvum)*	All	6	32	12.7-54.7
		AO	4	26.4	4.8-54.2
		AI	2	45.7	37.5-54.4
	Echinococcus multilocularis	All	13	3.3	1-6.5
		AO	2	1	0-1
		AI	11	4	1-8
	Giardia spp. (incl. G. intestinalis)	All	19	70.1	52-85.6
		AO	17	68.7	49.2-85.7
		AI	2	80.6	0-1
	Leptospira spp. (incl. L. interrogans)	All	9	22.2	9.7-37.8
		AO	2	29.6	7.6-55.7
		AI	7	20.5	7-37.6
	Toxoplasma gondii	All	6	24.3	15.6-34.3
		AO	2	37	0-84
		AI	4	20	12-29
	Trichinella spp.	All	2	1	0-3

		AO	1	0	
		AI	1	2	1-2
Nasua nasua	*Brucella spp.*	AO	2	6.8	0.9-16-5
	Leishmania spp. (incl. L. infantum)	AO	2	23.4	0-80.5
	Leptospira spp.	AO	2	13.4	0-47
	Trypanosoma evansi	AO	9	32.2	23.6-41.4
Nyctereutes procyonoides	*Borrelia spp.*	All	2	9.2	0-43.8
		AO	1	7	0-30
		AI	1	25	10.5-42.9
	Cryptosporidium spp. (incl. C. canis)	All	2	14	2-27
		AO	1	10.5	6.2-15.7
		AI	1	21.6	11.2-34
	Echinococcus multilocularis	All	12	4.5	2.5-7.1
		AO	1	23.1	3.6-50.4
		AI	11	4.1	2.2-6.5
	Francisella tularensis	All	2	12.7	9.4-16.3
		AO	1	9.5	0.2-26.6
		AI	1	12.8	9.4-16.5
	Lyssavirus Rabies lyssavirus	All	4	18.4	0-72
		AO	2	9.9	0-59.4
		AI	2	29	0-100
	Morbillivirus Canine morbillivirus	All	9	22.2	8.9-39
		AO	8	25.3	10.6-43.2
		AI	1	0	
	Protoparvovirus Carnivore protoparvovirus 1	All	3	20.8	0-55.9
		AO	2	31.6	0-74.5
		AI	1	6.1	0.1-17.4
	Sarcoptes scabiei	All	5	10.8	5.7-17.3
		AO	1	40.7	22.8-60
		AI	4	7.9	5.3-11.6
	Toxoplasma gondii	All	3	21.1	0-56.2
		AO	1	4.3	0-17.7
		AI	2	30.2	0-89.2
	Trichinella sp.	All	18	23.1	11.9-36.4
		AO	1	7.5	2.7-15
		AI	17	24.2	12.2-38.3
Procyon lotor	*Baylisascaris procyonis*	All	67	31.6	22.9-41
		AO	60	34.2	25.3-43.6
		AI	7	11.4	1.3-25
	Borrelia spp. (incl. B. burgdorferi, afzelii, garinii, turicatae, and lonestari)	All	20	16	8.3-25.7
		AO	17	21.5	12.4-32.1
		AI	3	0.4	0.1-0.9
	Campylobacter spp. (incl. C. jejuni)	All	3	12	0-36.2
		AO	2	19	0-63
		AI	1	1.3	0-3
	Coxiella burnetii	AO	2	9.6	2.7-19.5
	Cryptosporidium spp. (incl. C. parvum)	All	6	15.7	6.7-27.3
		AO	3	12.6	3.6-24.8
		AI	3	20.2	0-50
	Flavivirus Saint Louis encephalitis virus	AO	3	2.4	0-6.6
	Flavivirus spp.	AO	2	56.8	48.2-65
	Flavivirus West Nile Virus	AO	5	29.8	13.2-49.4
	Francisella tularensis	All	5	21.7	0-55.2
		AO	4	28.5	5.2-60.3
		AI	1	0.5	0.1-1.4

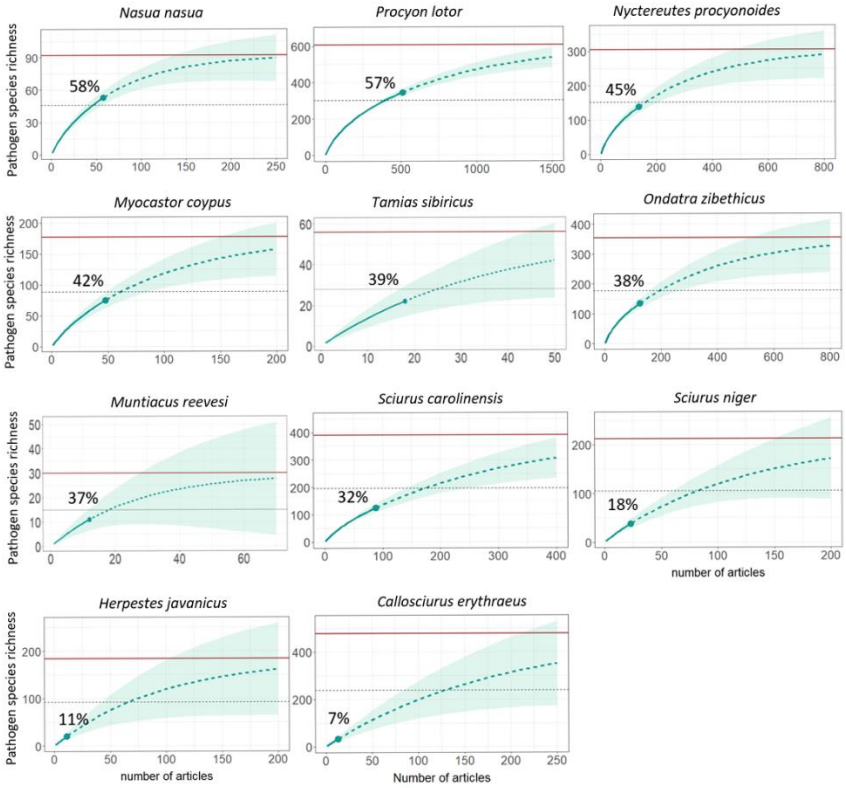
Influenzavirus A Influenza A virus (incl. H5N1)	All	3	1.6	0.8-2.7
	AO	1	2.4	1.3-3.6
	AI	2	1.3	0.5-2.4
Leptospira spp. (incl. L. borgpeterseni, interrogans, and kirshneri)	All	28	27.5	18.8-37.2
	AO	21	33.2	23.7-43.4
	AI	7	12.7	4.8-22.8
Lyssavirus Rabies lyssavirus	All	20	17.7	10.9-25.8
	AO	18	18.8	11.5-27.5
	AI	2	7.9	0-44.8
Mycobacterium bovis	AO	3	2.3	1.2-3.8
Morbillivirus Canine morbillivirus	All	19	27.5	18.7-37.3
	AO	13	2.9	18.5-40.3
	AI	6	24.5	4.6-48.6
Protoparvovirus sp.	All	3	51.8	5.4-96.4
	AO	2	72.6	22.6-100
	AI	1	12.4	6.3-19.2
Salmonella spp. (incl. S. enterica)	All	15	26.5	18.1-35.7
	AO	11	30.6	21.1-40.9
	AI	4	16.1	1.2-36.1
Toxoplasma gondii	All	33	38.6	29.5-48.1
	AO	24	46.8	34.9-58.9
	AI	9	18.4	9-29.5
Trichinella spp. (incl. T. spiralis, T. murreli, T. pseudospiralis, and t9)	All	22	5.6	3-8.8
	AO	16	7.4	3.5-12.3
	AI	6	1.6	2-3.8
Varicellovirus Suid alphaherpesvirus 1	All	4	2.5	0-13.2
	AO	3	3.5	0-18.8
	AI	1	0	
Yersinia spp. (incl. Y. pestis, Y. enterocoliica, and Y. pseudotubercolosis)	AI	2	18.9	0-62

Supplementary Material S6

Overall observed pathogen species richness, estimated pathogen species richness with confidence intervals (CI) and coverage of the observed pathogen species richness on the estimated one (%).

Host species	Observed pathogen species richness	Estimated pathogen species richness	95% C.I. lower	95% C.I. upper	Observed pathogen species richness on the estimated pathogen species richness (%)
Nasua nasua	53	92	69	147	**58**
Procyon lotor	345	606	520	733	**57**
Nyctereutes procyonoides	138	305	228	448	**45**
Myocastor coypus	75	177	123	293	**42**
Tamias sibiricus	22	56	32	143	**39**
Ondatra zibethicus	135	354	250	552	**38**
Muntjacus reevesi	11	30	15	107	**37**
Sciurus carolinensis	124	391	262	643	**32**
Sciurus niger	38	212	91	620	**18**
Herpestes javanicus	20	184	49	963	**11**
Callosciurus erythraeus	32	476	113	2472	**7**

Species accumulation curves of the pathogens (virus, bacteria, protozoa, helminths and ecto-parasites) of the eleven IAS analyzed. Red lines represents the curve asymptote, black dotted lines the 50% of the curve asymptote.



Chapter 4

Supplementary Material S1.

Questionnaire

The following table contains the questions (see "Question" column), along with the indications for the expert to provide their evaluations on the pathway events, including the risk factors to consider (see "Question additional information" column), the possible answers (see "Answers" column) and the related uncertainty levels (see "Uncertainty" column).
The first section (Questions Q1-Q5) is intended to frame the risk question, i.e. to define the IAS, area, target and the origin of the infectious disease risk of interest. The subsequent three sections follow the risk pathway steps reported in **Figure 1** of the manuscript /Supplementary Material S3: Hazard Identification (Questions Q6-Q10), Release assessment (Questions Q11-Q14), and Exposure assessment (Questions Q15-Q47). Refer to column "Apply to" to identify the questions specific for each target of interest.
Please, note that some questions are shown only if defined conditions are met (e.g., a certain type of answer to a previous question is given), and that the same question may have different formulations, details or answers according to the case. Refer to the columns "Conditions" (showing the conditions for which a certain answer is shown) and "Actions" (defining the following steps according to the answer given) to be guided in how to correctly read the questionnaire.

SECTION	Aim of the question	N	QUESTION	QUESTION ADDITIONAL INFORMATION	APPLY TO (target)	CONDITIONS	ANSWERS	ACTIONS	UNCERTAINTY
S1. CONTEXT	Define the IAS object of the risk assessment	1	Insert the latin name of the IAS for which you want to assess the disease risk		All		description		
	Define the geographic area object of the risk assessment	2	For which geographic area do you want to assess the disease risk of *the IAS*?		All		description		

SECTION	Aim of the question	N	QUESTION	QUESTION ADDITIONAL INFORMATION	APPLY TO (target)	CONDITIONS	ANSWERS	ACTIONS	UNCERTAINTY
	Define the target object of the risk assessment	3	For which target do you want to assess the disease risk of *the IAS*?		All		a. Humans b. Livestock c. Native wildlife		
	IAS current state in the area object of the risk assessment	4	Is *the IAS* already established in *the area*?		All		Yes No		
	Define the type of disease risk of interest	5	Are you interested in assessing risk related to new pathogens ("risk of introduction") or pathogens endemic to the area ("risk of amplification")?		All		a. Risk related to new pathogens b. Risk related to endemic pathogens c. Both	If ***a*** is selected, only pathogens not endemic to the are evaluated If ***b*** is selected, only pathogens endemic to the area are evaluated If ***c*** is selected, all pathogens are evaluated	
S2. HAZARD IDENTIFICATION	IAS susceptibility to the pathogen	6	Is the IAS susceptible to the following pathogens*? *Pathogens are shown according to target, list of pathogens is available in Supplementary Material S2	If information on the susceptibility of the IAS to the pathogen are not available, consider the phylogenetic proximity with other known host species for the pathogen.	All		a. Yes b. No	If **No** is selected, stop the risk assessment for the selected pathogens	
	Pathogen presence in *the area*	7	Are the following pathogens present in *the area*?		All	If the IAS is **already established** in the area (Q4=YES) The question applies to all pathogens to which the IAS is susceptible according to Q6	a. The pathogen is present and it has **not** been introduced by the IAS (endemic pathogen) b. The pathogen is present and it has been introduced by the IAS c. The pathogen is not present	Pathogens with answer **A** or **B** are evaluated for the risk of amplification and those with answer **C** for the risk of introduction	

SECTION	Aim of the question	N	QUESTION	QUESTION ADDITIONAL INFORMATION	APPLY TO (target)	CONDITIONS	ANSWERS	ACTIONS	UNCERTAINTY
		8	Are the following pathogens present in *the area*?		All	If the IAS **is not established** in the area (Q4=NO) The question applies to all pathogens to which the IAS is susceptible according to Q6	a. Yes b. No	Pathogens with answer **Yes** are evaluated for the risk of amplification, pathogens with answer **No** are evaluated for the risk of introduction	
	Target presence in *the area* The question identifies pathogens relevant to *the area* based on the presence of target species of interest	9	Is there a target for the following pathogens among the local livestock/ native wild species?		Livestock OR Native wildlife	If Q3=livestock OR native wildlife The question applies to all pathogens to which the IAS is susceptible according to Q6	a. Yes b. No	If **No** is selected, stop the risk assessment for the selected pathogens	
	Pathogen specific target	10	Select the main specific target/s for each pathogen in the area	E.g. Specific target for *Brucella abortus.* may be cows or camels according to the area	Livestock OR Native wildlife	If Q3=livestock OR native wildlife	Description		
S3. RELEASE ASSESSMENT	Likelihood of the IAS of being infected	11	How likely is *the IAS* established population of being infected with the following pathogens?	Suppose that the IAS has successfully established in *the area*. If known, refer to the region in the area where future introductions of the species are more likely to occur. Otherwise, refer to the worst case scenario. Consider: - The likely origin of the IAS (farm escape, introduction from another area where the pathogen is more or less widespread…)	All	If the IAS is **not** established in the area (Q4=NO) AND pathogen is **not** present (Q7=C)	High Medium Low Very low Negligible	If "Negligible" is selected, stop the risk assessment for the selected pathogens	High Medium Low

SECTION	Aim of the question	N	QUESTION	QUESTION ADDITIONAL INFORMATION	APPLY TO (target)	CONDITIONS	ANSWERS	ACTIONS	UNCERTAINTY
				- The area of release in terms of environmental conditions and availability/density of hosts and vectors					
		12	How likely is *the IAS* established population of being infected with the following pathogens?	Suppose that the IAS has successfully established in *the area*. If known, refer to the region in the area where future introductions of the species are most likely to occur. Otherwise, refer to the worst case scenario. Consider: - The likely origin of the IAS (farm escape, introduction from another area where the pathogen is more or less widespread…) - The area of release in terms of environmental conditions and availability/density of hosts and vectors - How widespread the pathogen is in the area, the presence of host species (both wild and domestic) that may enter in contact and transmit the pathogen to the IAS	All	If the IAS is **not** established in the area (Q4=NO), AND the pathogen **is** present (Q7=A OR B)	High Medium Low Very low Negligible	If "Negligible" is selected, stop the risk assessment for the selected pathogens	High Medium Low
		13	How likely is *the IAS* established population of being infected with the following pathogens?	Consider data available on the IAS established population or, in the absence of information: - The likely origin of the IAS (farm escape, introduction from another area where the pathogen is more or less widespread…) - The area of release in terms of environmental	All	If the IAS **is** established in the area (Q4=YES) AND the pathogen is **not** present (Q8=NO)	High (including the case in which there is evidence of infection in the IAS established population) Medium Low Very low Negligible (including the case in which there is evidence of pathogen absence in the IAS established population)	If "Negligible" is selected, stop the risk assessment for the selected pathogens	High Medium Low

SECTION	Aim of the question	N	QUESTION	QUESTION ADDITIONAL INFORMATION	APPLY TO (target)	CONDITIONS	ANSWERS	ACTIONS	UNCERTAINTY
				conditions and availability/density of hosts and vectors					
		14	How likely is *the IAS* established population of being infected with the following pathogens?	Consider data available on the IAS established population or, in the absence of information: - The likely origin of the IAS (farm escape, introduction from another area where the pathogen is more or less widespread…) - The area of release in terms of environmental conditions and availability/density of hosts and vectors - How widespread the pathogen is in the area, the presence of host species (both wild and domestic) that may enter in contact and transmit the pathogen to the IAS	All	If the IAS **is** established in the area (Q4=YES) AND the pathogen **is** present (Q8=YES)	High (including the case in which there is evidence of infection in the IAS established population) Medium Low Very low Negligible (including the case in which there is evidence of pathogen absence in the IAS established population)	If "Negligible" is selected, stop the risk assessment for the selected pathogens	High Medium Low
S4.EXPOSURE ASSESSMENT	Transmission from *the IAS* to WHC	15	Is there in *the area* a competent WHC able to transmit and possibly, maintain (i.e. act as reservoir), the following pathogens?		All If target = "local wildlife", "WHC" is intended as "WHC except target species"		a. A competent WHC able of transmission is present b. A competent WHC able of transmission and maintenance is present c. A competent WHC is not present	If **c** is selected skip this section (Q15-Q19) for the relative pathogens	High Medium Low
		16	Select the main likely route of transmission from *the IAS* to the competent WHC in *the area*		All	If 15C is not selected	a. Direct/very close contact, food (incl. predation/scavenging) b. Environmental contamination (water, air, soil, fomites) or mechanical vectors c. Vector-borne (biological vectors)	For pathogens where **a** is selected: answer to question 17 For pathogens where **b** is selected: answer to question 18	

SECTION	Aim of the question	N	QUESTION	QUESTION ADDITIONAL INFORMATION	APPLY TO (target)	CONDITIONS	ANSWERS	ACTIONS	UNCERTAINTY
								For pathogens where **c** is selected: answer to question 19	
		17	You selected **direct/very close contact or predation/scavenging** as the main likely route of transmission from *the IAS* to the local competent WHC for the following pathogens. How likely is transmission through this route?	Consider: - IAS and WHC structure in terms of density and distribution in the area - IAS and WHC behavioural patterns influencing pathogen spread (e.g. sociality, prey preferences) - IAS level of competence for intra and inter-species transmission and WHC susceptibility to pathogen	All	IF 16A is selected	High Medium Low Very low Negligible		High Medium Low
		18	You selected **environmental contamination (water, air, soil, fomites) or mechanical vectors** as the main likely route of transmission from *the IAS* to the local competent WHC for the following pathogens. How likely is transmission through this route?	Consider: - IAS and WHC structure in terms of density and distribution in the area - IAS and WHC behavioural patterns influencing pathogen spread (e.g. habitat preferences) - IAS level of competence and WHC susceptibility to pathogen - Local environmental characteristics that may influence pathogen spread (e.g. presence of wind, water, mechanical vectors)	All	IF 16B is selected	High Medium Low Very low Negligible		High Medium Low
		19	You selected **vector-borne** as the main likely route of transmission from *the IAS* to the local competent WHC for the following pathogens.		All	IF 16C is selected			
		19.1	How likely is transmission between *the IAS* and local vectors?	Consider: - the density of both IAS and vectors - vectors competence and host preferences - IAS susceptibility and competence	All	IF 16C is selected	High Medium Low Very low Negligible		High Medium Low

SECTION	Aim of the question	N	QUESTION	QUESTION ADDITIONAL INFORMATION	APPLY TO (target)	CONDITIONS	ANSWERS	ACTIONS	UNCERTAINTY
		19.2	How likely is transmission between local vectors and local WHC?	Consider: - the density of both competent WHC and vectors - vector competence and host preferences - WHC susceptibility and competence	All		High Medium Low Very low Negligible		High Medium Low
	Transmission from *the IAS* to DHC	20	Is there in *the area* a competent DHC able to transmit and possibly, maintain (i.e. act as reservoir), the following pathogens?		All If target = "livestock", "DHC" is intended as "DHC except target species"		a. A competent DHC able of transmission is present b. A competent DHC able of transmission and maintenance is present c. A competent DHC is not present	If c is selected skip this section (Q20-Q24) for the relative pathogens	
		21	Select the main likely route of transmission from *the IAS* to the competent DHC in *the area*.		All	If 20C is not selected	a. Direct/very close contact, food(incl. predation/scavenging) b. Environmental contamination (water, air, soil, fomites) or mechanical vectors c. Vector-borne (biological vectors)	For pathogens where a is selected: answer to question 22 For pathogens where b is selected: answer to question 23 For pathogens where c is selected: answer to question 24	
		22	You selected **direct/very close contact, food (incl. predation/scavenging)** as the main likely route of transmission from *the IAS* to the local competent DHC for the following pathogens. How likely is transmission through this route?	Consider: - IAS and DHC structure in terms of density and distribution in the area - DHC/IAS behavioural patterns influencing the spread of the pathogens (e.g. is the IAS a synanthropic species?) - IAS level of competence for intra and inter-species transmission and DHC susceptibility - Local animal management characteristics (e.g. extensive farming, access to pasture, biosecurity level)	All	IF 21A is selected	High Medium Low Very low Negligible		High Medium Low

SECTION	Aim of the question	N	QUESTION	QUESTION ADDITIONAL INFORMATION	APPLY TO (target)	CONDITIONS	ANSWERS	ACTIONS	UNCERTAINTY
		23	You selected **environmental contamination (water, air, soil, fomites) or mechanical vectors** as the main likely route of transmission from *the IAS* to the local competent DHC for the following pathogens. How likely is transmission through this route?	Consider: - IAS and DHC structure in terms of density and distribution in the area - DHC and IAS behavioural patterns influencing pathogen spread (e.g. is the IAS a synanthropic species?) - IAS level of competence for intra and inter-species transmission and DHC susceptibility - Local animal management characteristics (e.g. extensive farming, access to pasture, biosecurity level) - Local environmental characteristics that may influence pathogen spread (e.g. presence of wind, water, mechanical vectors)	All	IF 21B is selected	High Medium Low Very low Negligible		High Medium Low
		24	You selected **vector-borne** as the main likely route of transmission from *the IAS* to the local competent DHC for the following pathogens.		All	IF 21C is selected	High Medium Low Very low Negligible		High Medium Low
		24.1	How likely is the transmission between *the IAS* and the local vectors?	Consider: - the density of both IAS and vectors - vector competence and host preferences - IAS susceptibility and competence	All	IF 21C is selected	High Medium Low Very low Negligible		High Medium Low
		24.2	How likely is the transmission between local vectors and the local DHC?	Consider: - the density of both competent DHC and vectors - vector competence and host preferences - DHC susceptibility and competence	All	IF 21C is selected	High Medium Low Very low Negligible		High Medium Low
	Transmission from WHC to DHC	25	Select the main likely route of transmission from WHC to the competent DHC in *the area*		All	If both WHC and DHC are present in the area	a. Direct/very close contact, food (incl. predation/scavenging) b. Environmental contamination (water, air, soil, fomites) or mechanical vectors	For pathogens where a is selected: answer to question 26	

SECTION	Aim of the question	N	QUESTION	QUESTION ADDITIONAL INFORMATION	APPLY TO (target)	CONDITIONS	ANSWERS	ACTIONS	UNCERTAINTY
							c. Vector-borne (biological vectors)	For pathogens where b is selected: answer to question 27 For pathogens where c is selected: answer to question 28	
		26	You selected **direct/very close contact, food (incl. predation/scavenging)** as the main likely route of transmission from WHC to DHC for the following pathogens. How likely is transmission through this route?	Consider: - DHC and WHC structure in terms of density and distribution in the area - DHC level of competence for intra and inter-species transmission and WHC susceptibility to the pathogens - DHC and WHC behavioural patterns that may influence pathogen spread to the DHC (e.g. does the WHC include synanthropic species?) - local animal management characteristics (e.g. extensive farming, access to pasture, biosecurity level, vaccination programs)	All If target = "livestock" OR "local wildlife", "DHC"/"WHC" is intended as "DHC except target species"/ "WHC except target species"	If 25A is selected	High Medium Low Very low Negligible		High Medium Low
		27	You selected **environmental contamination (water, air, soil, fomites) or mechanical vectors** as the main likely route of transmission from WHC to DHC for the following pathogens. How likely is transmission through this route?	Consider: - DHC and WHC structure in terms of density and distribution in the area - WHC level of competence for intra and inter-species transmission and DHC susceptibility to the pathogens - WHC and DHC behavioural patterns that may influence pathogen spread to the DHC (e.g. does the WHC include synanthropic species?) - local animal management characteristics (e.g. extensive farming, access to pasture, biosecurity level, vaccination programs) - Local environmental character-	All	If 25B is selected	High Medium Low Very low Negligible		High Medium Low

SECTION	Aim of the question	N	QUESTION	QUESTION ADDITIONAL INFORMATION	APPLY TO (target)	CONDITIONS	ANSWERS	ACTIONS	UNCERTAINTY
				istics that may influence pathogen spread (e.g. presence of wind, water, mechanical vectors)					
		28	You selected **vector-borne** as the main likely route of transmission from WHC to the local competent DHC for the following pathogens.		All	If 25C is selected			
		28.1	How likely is the transmission between local vectors and the local WHC?	Consider: - the density of both competent WHC and vectors - vector competence and host preferences - WHC susceptibility and competence	All		High Medium Low Very low Negligible		High Medium Low
		28.2	How likely is transmission between local vectors and the local DHC?	Consider: - the density of both competent DHC and vectors - vector competence and host preferences - DHC susceptibility and competence	All		High Medium Low Very low Negligible		High Medium Low
	Transmission from DHC to WHC	29-32	Repeat questions 25-28 by changing the directionality of the transmission and related factors		All	If both WHC and DHC are present in the area			
	Transmission from IAS to TARGET	33	Select the main likely route of transmission from *the IAS* to *the target* in *the area*		Human target		a. Direct/very close contact b. Environmental contamination (water, air, soil, fomites) or mechanical vectors c. Food-borne d. Vector-borne (biological vectors)	For pathogens where **a** is selected: answer to question 34 For pathogens where **b** is selected: answer to question 35 For pathogens where **c** is selected: answer to question 36 For pathogens where **d** is selected: answer to question 37	

SECTION	Aim of the question	N	QUESTION	QUESTION ADDITIONAL INFORMATION	APPLY TO (target)	CONDITIONS	ANSWERS	ACTIONS	UNCERTAINTY
					Livestock/wildlife target		a. Direct/very close contact, food (incl. predation/scavenging) b. Environmental contamination (water, air, soil, fomites) or mechanical vectors c. Vector-borne (biological vectors)	For pathogens where **a** is selected: answer to question 34 For pathogens where **b** is selected: answer to question 35 For pathogens where **c** is selected: answer to question 37	
		34	You selected **Direct/very close contact** as the main likely route of transmission from **IAS** to *the target* for the following pathogens. How likely is transmission through this route?	Consider: **-**IAS level of competence for intra and inter-species transmission -IAS behavioural patterns that influence pathogen spread (e.g. is the IAS a synanthropic species?) -human occupational or recreational activities (e.g. encroachment of human settlements into sylvatic areas, outdoor activities, local customs, sanitary conditions)	Human target	If 33A is selected	High Medium Low Very low Negligible		High Medium Low
			You selected Direct/very close contact, food (incl. predation/scavenging) as the main likely route of transmission from **IAS** to *the target* for the following pathogens. How likely is transmission through this route?	Consider: - IAS and livestock target structure in terms of density and distribution in the area - IAS level of competence for intra and inter-species transmission and livestock target susceptibility to the pathogens - IAS and livestock target behavioural patterns that may influence pathogen spread to the DHC (e.g. is the IAS a synanthropic species?) - local animal management characteristics (e.g. extensive farming, access to pasture, biosecurity level, vaccination programs)	Livestock target				

SECTION	Aim of the question	N	QUESTION	QUESTION ADDITIONAL INFORMATION	APPLY TO (target)	CONDITIONS	ANSWERS	ACTIONS	UNCERTAINTY
				Consider: - IAS and wildlife target structure in terms of density and distribution in the area - IAS and wildlife target behavioural patterns influencing pathogen spread (e.g. sociality, prey preferences); - IAS level of competence for intra and inter-species transmission and wildlife target susceptibility to pathogen	Wildlife target				
		35	You selected **Environmental contamination (water, air, soil, fomites) or mechanical vectors** as the main likely route of transmission from IAS to *the target* for the following pathogens. How likely is transmission through this route?	Consider: - IAS level of competence for intra and inter-species transmission - IAS behavioural patterns that influence pathogen spread (e.g. is the IAS a synanthropic species?) - Human occupational or recreational activities (e.g. encroachment of human settlements into sylvatic areas, outdoor activities, local customs, sanitary conditions) - Local environmental characteristics that may influence pathogen spread (e.g. presence of wind, water, mechanical vectors)	Human target	If 33B is selected	High Medium Low Very low Negligible		High Medium Low
				Consider: - IAS level of competence for intra and inter-species transmission - IAS behavioural patterns that influence pathogen spread (e.g. is the IAS a synanthropic species?) - Local animal management characteristics (e.g. extensive farming, access to pasture, biosecurity level, vaccination programs) - Local environmental characteristics that may influence pathogen spread (e.g. presence of wind, water, mechanical vectors)	Livestock target				

SECTION	Aim of the question	N	QUESTION	QUESTION ADDITIONAL INFORMATION	APPLY TO (target)	CONDITIONS	ANSWERS	ACTIONS	UNCERTAINTY
				Consider: - IAS level of competence for intra and inter-species transmission - IAS behavioural patterns that influence pathogen spread - Local environmental characteristics that may influence pathogen spread (e.g. presence of wind, water, mechanical vectors)	Wildlife target				
		36	You selected **food-borne** as the main likely route of transmission from *the IAS* to *the target* for the following pathogens. How likely is transmission through this route?	Consider: -local customs and practices, sanitary conditions, biosecurity levels	Human target	If 33C is selected	High Medium Low Very low Negligible		High Medium Low
		37	You selected **vector-borne** as the main likely route of transmission from *the IAS* to *the target* for the following pathogens		All	If 33D (for human target) OR 33C (for other targets) is selected			
		37.1	How likely is the transmission between IAS and the local vectors?	Consider: - the density of both IAS and vectors - vector competence and host preferences - IAS susceptibility and competence	All		High Medium Low Very low Negligible		High Medium Low
		37.2	How likely is the transmission between local vector and *the target*?	Consider: - vector density and competence - environmental characteristics and land use	Human target		High Medium Low Very low Negligible		High Medium Low
				Consider: - the density of both *the target* and vectors - vector competence and host preferences - *the target* susceptibility	Livestock and wildlife target				
	Transmission from WHC to TARGET	38-42	Repeat Q33-37			If WHC is present			

SECTION	Aim of the question	N	QUESTION	QUESTION ADDITIONAL INFORMATION	APPLY TO (target)	CONDITIONS	ANSWERS	ACTIONS	UNCERTAINTY
	Transmission from DHC to TARGET	43	Select the main likely route of transmission from **DHC** to *the target* in *the area*		Human target	If DHC is present	a. Direct/very close contact b. Environmental contamination (water, air, soil, fomites) or mechanical vectors c. Food-borne d. Vector-borne (biological vectors)	For pathogens where **a** is selected: answer to question 44 For pathogens where **b** is selected: answer to question 45 For pathogens where **c** is selected: answer to question 46 For pathogens where **d** is selected: answer to question 47	
					Livestock/wildlife target		a. Direct/very close contact, food (incl. predation/scavenging) b. Environmental contamination (water, air, soil, fomites) or mechanical vectors c. Vector-borne (biological vectors)	For pathogens where **a** is selected: answer to question 44 For pathogens where **b** is selected: answer to question 45 For pathogens where **c** is selected: answer to question 47	
		44	You selected **Direct/very close contact** as the main likely route of transmission from DHC to *the target* for the following pathogens. How likely is transmission through this route?	Consider: -DHC level of competence for intra and inter-species transmission -local customs (e.g. raw milk consumption) and practices in animal husbandry, sanitary conditions, biosecurity levels	Human target	If 43A is selected	High Medium Low Very low Negligible		High Medium Low

SECTION	Aim of the question	N	QUESTION	QUESTION ADDITIONAL INFORMATION	APPLY TO (target)	CONDITIONS	ANSWERS	ACTIONS	UNCERTAINTY
			You selected **Direct/very close contact, food (incl. predation/scavenging)** as the main likely route of transmission from **IAS** to *the target* for the following pathogens. How likely is transmission through this route?	Consider: - DHC and livestock target structure in terms of density and distribution in the area - DHC level of competence for intra and inter-species transmission and livestock target susceptibility to the pathogens - DHC and livestock target behavioral patterns that may influence pathogen spread to the target - local animal management characteristics (e.g. multi-species farming, extensive farming, access to pasture, biosecurity level, vaccination programs)	Livestock target				
				Consider: - DHC and wildlife target structure in terms of density and distribution in the area - DHC level of competence for intra and inter-species transmission and wildlife target susceptibility to the pathogens - DHC and wildlife target behavioural patterns that may influence pathogen spread - local animal management characteristics (e.g. extensive farming, access to pasture, biosecurity level, vaccination programs)	Wildlife target				
		45	You selected **Environmental contamination (water, air, soil, fomites) or mechanical vectors** as the main likely route of transmission from IAS to *the target* for the following pathogens. How likely is transmission through this route?	Consider: - DHC level of competence for intra and inter-species transmission - DHC behavioural patterns that influence pathogen spread - Human occupational or recreational activities (e.g. encroachment of human settlements into sylvatic areas, outdoor activities, local customs, sanitary conditions) - Local environmental character-	Human target	If 43B is selected	High Medium Low Very low Negligible		High Medium Low

SECTION	Aim of the question	N	QUESTION	QUESTION ADDITIONAL INFORMATION	APPLY TO (target)	CONDITIONS	ANSWERS	ACTIONS	UNCERTAINTY
				istics that may influence pathogen spread (e.g. presence of wind, water, mechanical vectors)					
				Consider: - DHC level of competence for intra and inter-species transmission and livestock target susceptibility to the pathogens - DHC behavioural patterns that influence pathogen spread - Local animal management characteristics (e.g. extensive farming, access to pasture, biosecurity level, vaccination programs) - Local environmental characteristics that may influence pathogen spread (e.g. presence of wind, water, mechanical vectors)	Livestock target				
				Consider: - DHC level of competence for intra and inter-species transmission and wildlife target susceptibility to the pathogens - Wildlife target behavioural patterns that influence pathogen spread - Local environmental characteristics that may influence pathogen spread (e.g. presence of wind, water, mechanical vectors)	Wildlife target				
		46	You selected **food-borne** as the main likely route of transmission from DHC to *the target* for the following pathogens. How likely is transmission through this route?	Consider: -local customs and practices, sanitary conditions, biosecurity levels	Human target	If 43C is selected	High Medium Low Very low Negligible		High Medium Low
		47	You selected **vector-borne** as the main likely route of transmission from *DHC* to *the target* for the following pathogens.			If 43D (for human target) or 43C (for other targets) is selected	High Medium Low Very low Negligible		High Medium Low

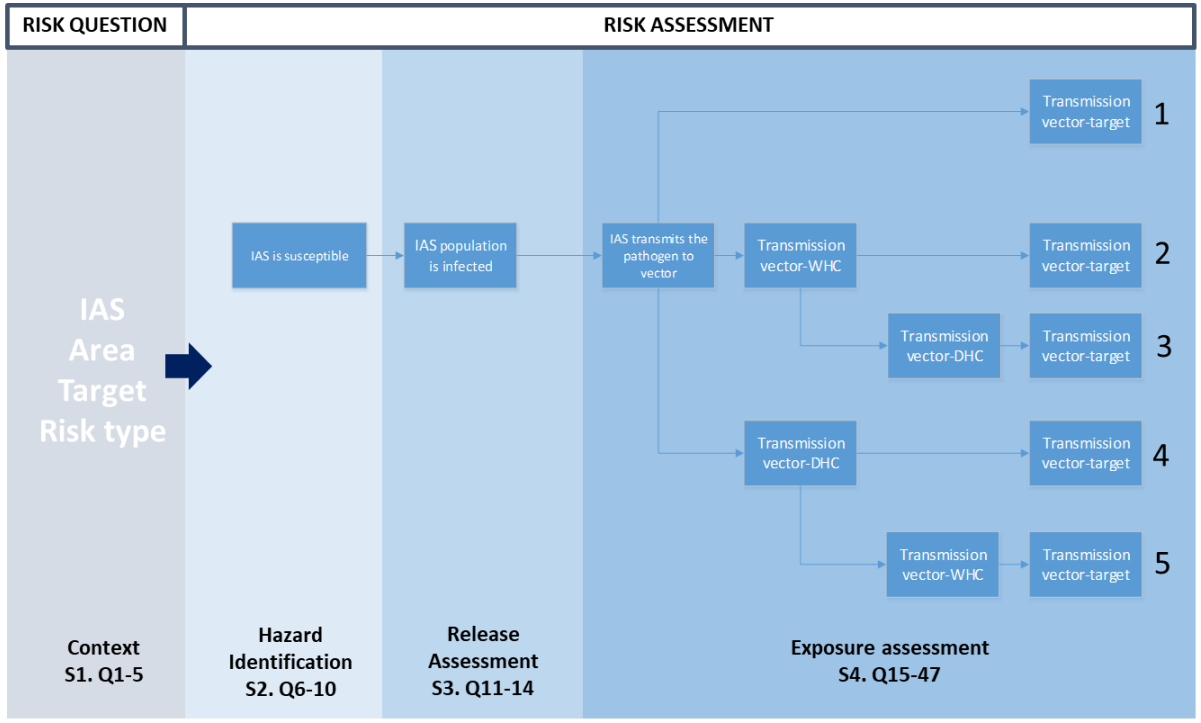
SECTION	Aim of the question	N	QUESTION	QUESTION ADDITIONAL INFORMATION	APPLY TO (target)	CONDITIONS	ANSWERS	ACTIONS	UNCERTAINTY
		47.1	How likely is the transmission between DHC and the local vectors?	Consider: - the density of both competent DHC and vectors - vector competence and host preferences - DHC susceptibility and competence	All		High Medium Low Very low Negligible		High Medium Low
		47.2	How likely is the transmission between local vector and *the target*?	Consider: - vector density and competence in the transmission of the pathogen - environmental characteristics and land use	Human target		High Medium Low Very low Negligible		High Medium Low
				Consider: - the density of both *the target* and vectors - vector competence and host preferences - *the target* susceptibility	Livestock and wildlife target				

Supplementary Material S2.

Vector-borne pathogens pathway

Pathway for vector-borne pathogen, where target can be alternatively considered humans, domestic animals, and wildlife. Five sub-pathways have been identified in the process: (1) Transmission IAS-vector-target, (2) Transmission IAS-vector-WHC-vector-target, (3) Transmission IAS-vector-WHC-vector-DHC-vector-target, (4) Transmission IAS-vector-DHC-vector-target, and (5) Transmission IAS-vector-DHC-vector-WHC-vector-target.

IAS=Invasive Alien Species, WHC=Wild Host Community, DHC= Domestic Host Community.



Supplementary Material S3.

Risk estimates obtained for *P. lotor* and *S. carolinensis* in the application test related to the five sub-pathways applying the matrix described in the article, and overall risk estimates obtained combining the risk estimates of the sub-pathways according to the method described in Chapter 5.
Pathogens that have been evaluated for the risk of introduction are underlined, the remaining pathogens have been evaluated for the risk of amplification.

IAS=Invasive Alien Species
WHC=Wild Host Community
DHC=Domestic Host Community

HOST: *PROCYON LOTOR*

	IAS→Humans		**IAS→WHC→Humans**		**IAS→WHC→DHC→Humans**		**IAS→DHC→Humans**		**IAS→DHC→WHC→Humans**		**Overall risk**	
	Risk	**Uncer-tainty**	**Risk**	**Uncertainty**	**Risk**	**Uncertainty**	**Risk**	**Uncertainty**	**Risk**	**Uncertainty**	**Risk**	**Uncertainty**
Bacillus anthracis											Negligible	Low
Baylisascaris procyonis											Negligible	Low
Borrelia burgdorferi sl	Medium	Medium	Medium	Medium	NA	NA	NA	NA	NA	NA	High	Medium
Chikungunya virus											Negligible	Low
Clostridium botulinum											Negligible	Low
Corynebacterium diphtheriae, C. ulcerans, C. pseudotubercu-losis											Negligible	Medium
Coxiella burnetii	Very low	Medium	Negligible	Medium	Negligible	Medium	Negligible	Medium	Negligible	Medium	Very low	Medium
Creutzfeldt-Jakob disease											NA	NA
Cryptosporidium spp.	Very Low	Low	Very Low	Low	Low	Low	Very Low	Low	Very low	Medium	Medium	Medium
Dengue virus											Negligible	Low
E. coli (STEC/VTEC)											NA	NA
Echinococcus multilocularis, E. granolosus											Negligible	Low

Giardia lamblia	Very Low	Low	Very Low	Low	Low	Low	Low	Low	Very low	Low	Medium	Low
Hepatitis B virus											NA	NA
Human pathogenic Brucella spp.	Negligible		Negligible		Negligible		Negligible		Negligible		Negligible	Medium
Human pathogenic Campylobacter spp.	Very Low	Low	Very Low	Medium	Medium	Medium	Low	Low	Very Low	Medium	High	Medium
Influenza virus A/H5											Negligible	Medium
Leptospira interrogans or any other pathogenic Leptospira	Negligible	Medium	Low	Medium	Very Low	Medium	Very Low	Medium	Negligible	Medium	Medium	Medium
Listeria monocytogenes											Negligible	Medium
Mycobacterium tubercolosis complex											Negligible	Low
Plasmodium spp.											NA	NA
Rabies											Negligible	Low
Salmonella enteritis	Very Low	Medium	Very Low	Medium	Low	Medium	Medium	Medium	Very low	Medium	High	Medium
Salmonella typhi, S. paratyphi											Negligible	Medium
*Severe acute respiratory syndrome (SARS)**											Negligible	High
Shigella spp.											Negligible	Medium
Tick-borne encephalitis virus	Low	Medium	Low	Medium	Low	Medium	Low	Medium	Low	Medium	High	Medium
Toxoplasma gondii	Negligible	Low	Negligible	Low	Negligible	Low	Negligible	Low	Negligible	Low	Negligible	Low
Trichinella	Negligible	Medium	Very low	Medium	Very low	Medium	Negligible	Medium	Negligible	Medium	Low	Medium
Vibrio cholerae											NA	NA
Viral haemorrhagic fevers (VHF)											NA	NA
West Nile Virus											Negligible	Medium
Yellow fever virus											Negligible	Low
Yersinia enterocolitica, Y. pseudotuberculosis											NA	NA
Yersinia pestis											NA	NA
Zika virus											Negligible	Low

*This assessment evaluated the risk related to Sars-Cov-1

HOST: *SCIURUS CAROLINENSIS*

	IAS→Humans		IAS→WHC→Humans		IAS→WHC→DHC→Humans		IAS→DHC→Humans		IAS→DHC→WHC→Humans		Overall risk	
	Risk	**Uncertainty**	**Risk**	**Uncertainty**	**Risk**	**Uncertainty**	**Risk**	**Uncertainty**	**Risk**	**Uncertainty**	**Risk**	**Uncertainty**
Bacillus anthracis											Negligible	Low
Borrelia burgdorferi	Low	Medium	Low	Medium	NA	NA	NA	NA	NA	NA	Medium	Medium
Chikungunya virus											Negligible	Medium
Clostridium botulinum											Negligible	High
Corynebacterium diphtheriae, C. ulcerans, C. pseudotuberculosis											Negligible	Medium
Coxiella burnetii											Negligible	Medium
Creutzfeldt-Jakob disease											NA	NA
Cryptosporidium spp.	Negligible	Low	Negligible	Medium	Negligible	Medium	Negligible	Low	Negligible	Medium	Negligible	Medium
Dengue virus											Negligible	Low
E. coli (STEC/VTEC)											NA	NA
Echinococcus multilocularis, E. granolosus											Negligible	Low
Giardia lamblia	Negligible	Medium	Negligible	Medium	Negligible	Medium	Low	Medium	Very low	Medium	Low	Medium
Hepatitis B virus											NA	NA
Human pathogenic Brucella spp.											Negligible	Medium
Human pathogenic Campylobacter spp.	Negligible	Medium	Negligible	Medium	LNegligible	Medium	Negligible	Medium	Negligible	Medium	Negligible	Medium
Influenza virus A/H5N1											Negligible	Medium
Leptospira spp.											Negligible	Low
Listeria monocytogenes											Negligible	Medium
Ljungan virus	Negligible	Low	Negligible	High	NA	NA	NA	NA	NA	NA	Negligible	High

Mycobacterium tubercolosis											Negligible	Low
Plasmodium spp.											NA	NA
Rabies virus											Negligible	Low
Salmonella enteritis	Negligible	Medium	Negligible	Medium	Negligible	Medium	Negligible	Medium	Negligible	Medium	Negligible	Medium
Salmonella typhi, S. paratyphi											Negligible	Low
*Severe acute respiratory syndrome (SARS)**											Negligible	Medium
Shigella spp.											NA	NA
TBE											Negligible	Low
Toxoplasma gondii	Negligible	Low	Low	Medium	Low	Medium	Very Low	Medium	Very Low	Medium	Medium	Medium
Trichinella											Negligible	Low
Vibrio cholerae											NA	NA
Viral haemorrhagic fevers (VHF)											NA	NA
West Nile Virus	Very low	Medium	Very low	Medium	Very low	High	Very low	High	Very low	High	Medium	High
Yellow fever virus											Negligible	Low
Yersinia enterocolitica, Y. pseudotuberculosis	Negligible	Medium	Negligible	Medium	Negligible	Medium	Negligible	Medium	Negligible	Medium	Negligible	Medium
Yersinia pestis											Negligible	Low
Zika Virus											Negligible	Low

*This assessment evaluated the riskelated to Sars-Cov-1

AKNOWLEDGEMENTS

A special thanks to my tutor, Prof. Nicola Ferrari, for giving me the opportunity to carry out this project, and for always remembering me the importance to “walk”.

Thanks to Matteo Crotta, for having introduced and accompanied me in the world of risk analysis.

Thanks to Prof. Javier Guitian, for having hosted me in its Department at the Royal Veterinary College, an experience that was relevant for both my personal and professional growth.

Thanks to the experts involved in the project for their time and comments.

And lastly, thanks to all my collegues of the lab, for making my PhD experience much richer.