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Detection of zoonotic pathogens in animals performed at the University Hospital Institute Méditerranée Infection (Marseille – France)

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ABSTRACT

At the University Hospital Institute Méditerranée Infection (IHU, Marseille, France), for almost thirty years, veterinarians have been carrying out epidemiological investigations, together with doctors, on animals living near human cases of zoonoses, on the one hand, and on the other hand, transverse and longitudinal epidemiological surveillance studies on animals which are reservoirs, vectors or sentinels of potentially zoonotic infections,. This article presents the methods adopted and the results obtained from these studies. They have been the subject of 76 peer-reviewed publications relating to wild animals (37 publications) and/or domestic animals (48 publications). These studies were often carried out in the field with veterinarians from the French army's health service (39 publications). They were at the origin of the detection of some thirty zoonotic pathogens in the laboratories of the IHU (64 publications) and/or other French laboratories (18 publications). Our approach is an original embodiment of the "One Health" concept.

1. Introduction

The most serious contemporary pandemics and epidemics with pandemic potential (HIV, influenza, SARS-2003, MERS-CoV, Covid-19) are due to RNA viruses that evolve from viruses naturally hosted by animals [1,2]. More than 60% of the infectious diseases known to humans have an animal reservoir. This is also the case for some 75% of emerging diseases and 80% of bioterrorism agents. It is clear that most human infectious diseases originate from animals [1,3–5]. In this context, it makes sense to have a veterinary component within the context of the University Hospital Institute (IHU for *Institut Hospitalo-Universitaire*) Méditerranée Infection (Marseille, France). It is a clear illustration of the relevance of the "One Health" concept. In this article, we shall explain how an operational epidemiological surveillance system for reservoir, vector and/or sentinel animals of human infections was developed from field to laboratory within the IHU. We shall then present an overview of all the studies carried out and published following this strategy.

2. Methodology

2.1. Field surveys

Several types of epidemiological surveillance surveys on infectious diseases in animals were carried out in a wide variety of contexts as a regular part of the IHU's research activities. The creation of a veterinary unit within a hospital establishment is a rare event and this multidisciplinary approach is one of the IHU's strengths. First, investigations were carried out on animals living in the environment of patients suspected of having zoonoses, at the request of physicians specialized in infectious diseases (Fig. 1). Domestic animals (dogs, cats, etc.) were the first to be sampled, soon followed by commensal animals (rats, birds, etc.) and others living in or near the environment of the patients. Surveys were thus performed out in localities where an outbreak of several grouped cases of zoonoses had been reported (e.g. Q fever). These field studies required material preparation to facilitate the representative sampling to be carried out according to the various opportunities and epidemiological contexts. If field samples are to be optimal, quantitatively and

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qualitatively, they must be collected in the right place, at the right time, on suitable species and matrices and using the most appropriate techniques. The epidemiological findings hit upon by the IHU using this method were inevitably serendipitous, but they are the results of more than forty years of field experience. The Veterinary Research Center (VRC), an annex of the IHU, is equipped to initiate or receive biological samples (blood, faeces, swabs, biopsies, etc.), endoparasites and ectoparasites of animal origin. For the ectoparasites, the VRC maintains close functional relations with the entomologists of the IHU insectarium. The VRC can also accommodate animals, domestic or wild, living or dead, suspected of zoonotic or unknown infectious diseases or likely to be asymptomatic carriers of pathogenic agents. Ante- or post-mortem diagnostic sampling can also be carried out on these animals there. The design of the VRC's facilities and the management of flows have been designed to carry out these activities in accordance with all the required biosafety rules, both within the IHU's premises and outside these premises. Another aspect of the veterinary epidemiosurveillance carried out at the IHU concerns cross-sectional and longitudinal studies carried out in targeted geographical areas at risk for certain zoonoses (e.g. African villages). Various samples (blood, rectal swabs) were taken from various domestic animals. These samples were then screened according to different screening techniques (serology, PCR, culture), in order to detect a pattern of infectious agents with zoonotic potential. The skills currently deployed within the IHU VRC in terms of epidemiological surveillance and field epidemiology are the fruit of many years of experience acquired in the context of epidemiological activities carried out on dogs and horses for the French Army and the National Gendarmerie. These latter sometimes acted as sentinels, making it possible to identify the animal or human disease agents circulating in certain ecosystems (e.g. Rift Valley fever, West Nile fever, dirofilariosis, leishmaniosis, trypanosomosis, etc.). Army veterinarians routinely drew the blood of military dogs, staying outside mainland France on missions, before their departure and on their return. These samples were fed into a sample bank, the use of which provided valuable information on exposure to certain pathogens in areas where the armed forces are deployed.

2.2. *Ethical approval, biodiversity conservation, benefit-sharing obligations, and biosafety*

All sampling of live animals (e.g. blood) is carried out in accordance with the international standards both for animal welfare [6] and for the preservation of protected species (CITES -Washington convention) [7], with European regulations [8] and the regulations of the country where the samples are collected. Where necessary, particularly with wild animals, chemical restraint techniques (teleanesthesia) are used, in accordance with the rules of good veterinary practice. This was the case for example, when for the first time in the history of research, sperm was collected from green monkeys by means of an electroejaculation technique using a rectal probe specially designed for our study. In addition, the IHU VRC's collaborative network with wildlife veterinarians provided access to samples (blood, swabs) collected from wild animals anaesthetised for various reasons: in zoological parks (for example, in the case of injuries) or during transport (in the case of bears from Slovenia introduced into the Pyrenees). The majority of animal samples collected in the course of the studies conducted by the IHU were collected in a non-invasive manner (e.g. faeces). Animals that had died recently, either in accidental circumstances (animals fatally injured on the road found during the mission) or as part of official operations carried out within the study area (hunting, slaughterhouse products, markets, campaigns to destroy pests such as rodents, etc.) were also frequently collected. Older remains (bones, teeth, coprolites, skins) could sometimes be collected in the wild (mass graves) or even in museums. Similarly, the concern to preserve biodiversity is increasingly taken into account, particularly since the adoption of the Convention on Biodiversity by the United Nations at a world conference in Nagoya (Japan) in 2010 [9]. France, as most of the countries of origin from which the animal samples were collected for studies carried out at the

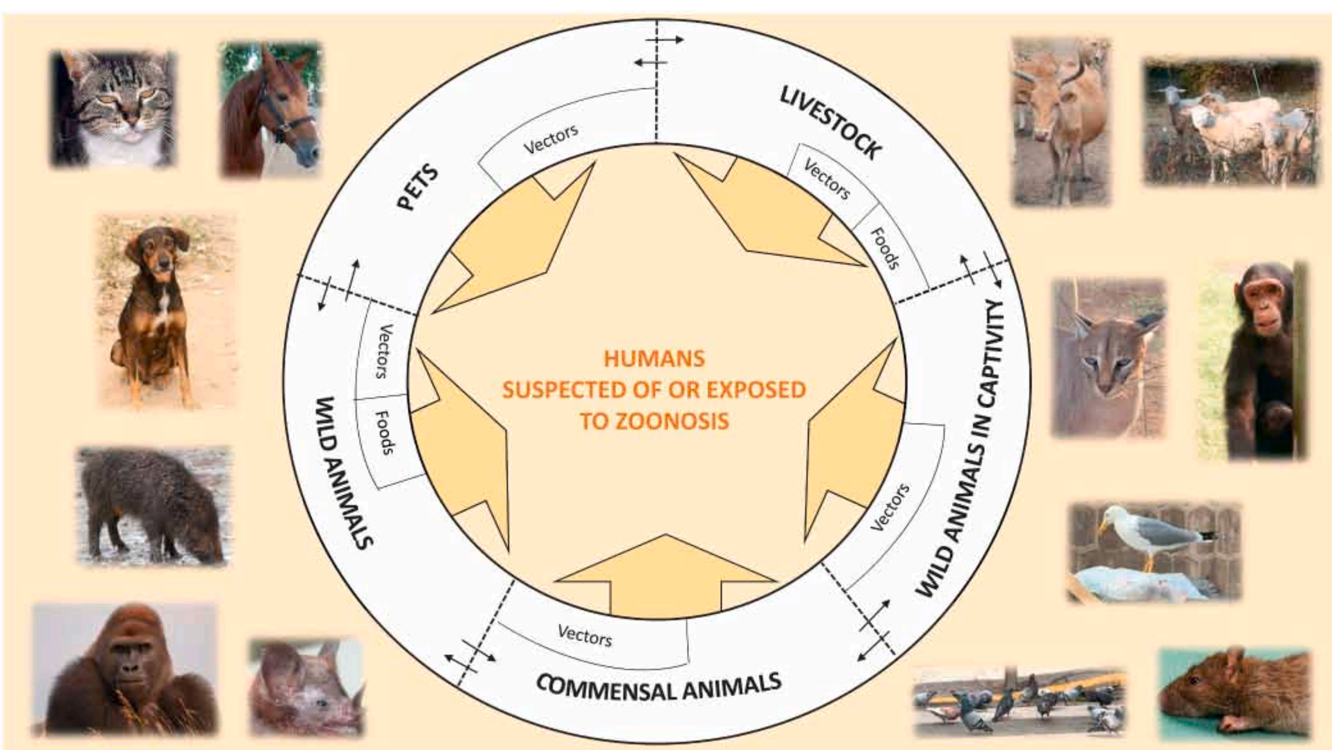

Fig. 1. The variety of reservoir animals, sentinels or potential vectors in the entourage of a human person who is sick, suspected of and/or exposed to, zoonoses (Pictures: B. Davoust).

IHU, has ratified the Nagoya Protocol on access to genetic resources and fair and equitable sharing of the benefits arising out of their utilization. We are committed, on the one hand, never to participate in the destruction of endangered species of animals and, on the other hand, not to appropriate illegitimately any samples of animal origin (biopiracy). Whenever we conduct a study in a foreign country, we collaborate with the local scientists. These are partnerships that are rewarding for all parties involved. All animal samples collected within the framework of IHU activities are systematically subject to prior authorization from the competent national authorities (often the Ministry of the Environment), as well as from local authorities (e.g. village chiefs) and the owners of the animals collected, in the interests of transparency and good mutual relations. With regard to biosafety, we apply protocols to ensure that infectious diseases are not introduced from one country to another. Before shipment, official authorisation is obtained for the import of samples of animal origin for diagnosis and scientific research from the Bouches-du-Rhône (Marseille) prefecture in agreement with European regulations [10]. The samples are then transported to the IHU laboratories in accordance with current air transport standards [11]. Field staff working with the animals, as well as all those working on the samples, have received training in biosecurity. Several vaccinations (against rabies, leptospirosis …) and chemoprevention may be recommended.

2.3. Collections

For more than twenty years, more than a hundred epidemiological surveys have been conducted in 25 countries on five continents, mainly in Africa. In total, more than 35,000 biological samples of animal origin, in addition to ectoparasite specimens, have been collected. An aliquot of each sample was preserved after analysis using various techniques (mainly freezing, alcohol, desiccation, etc.), thus constituting an important sample bank. Thousands of samples of faeces, sera, blood, internal organs (spleen, liver, kidneys, lungs, intestines, etc.), muscles, skin, teeth, etc. are also available in this bank. Samples from the domestic dog (*Canis lupus*) are the most represented. The bank also has nearly 17,000 blood samples from French military working dogs, collected over the last thirty years, particularly in the context of missions outside mainland France (Africa, Afghanistan, French Guiana, the Middle East, etc.). The other half of the samples were taken mainly from mammals of some 120 different species, both domestic ($N = 30$) and wild.

3. Investigations conducted on wild animals

3.1. Non-human primates

Non-human primates (NHPs), particularly the great apes (gorillas, chimpanzees, orangutans), are the animals that are phylogenetically closest to humans, with whom they share the greatest number of diseases. NHPs are wild animals that may have occasional contact with humans, for example in the course poaching activities, where pathogens may be transmitted to humans and can cause potential emerging diseases [12]. A synthesis of studies conducted at the IHU, using mainly faeces from great apes, has been published recently [13]. Thus, human adenovirus genotypes were discovered in gorillas from Congo-Brazzaville and, conversely, simian genotypes have been found in human beings sharing the same living area as the gorillas [14]. Again, a recombinant coxsackie/poliovirus has been found in wild gorillas and in the guardians of the reserve. One gorilla in fact presented sequelae of a central nervous system infection (muscular atrophies and flaccid paralysis) possibly due to this virus [15]. Work continues on the venereal infection of NHPs by the yaws agent (*Treponema pallidum* subsps. pertenue) [16,17] as well as on the detection of antibiotic-resistant bacteria in the faeces of these primates. NHPs share with humans many protozoan infections [18]. Table 1 provides additional information on the studies published within the framework of IHU activities [19–23]. They concern eleven NHPs species from Africa and French Guiana.

3.2. Bats

Bats are responsible for the transmission of many zoonoses, including the SARS-CoV-2 infection [2]. The ecology of numerous chiropteran species, in particular their extreme promiscuity within colonies often of several thousand bats, represents a major risk of transmission of pathogens to humans, either directly or via intermediate hosts [24]. These bat reservoirs are very effective because they are highly resistant to infection. A bat capture-release campaign was carried out as part of the IHU studies in French Guiana in search of the wild reservoir(s) of the causative agent of human Q fever (*Coxiella burnetii*). Blood samples and swabs were taken from 283 individuals from 21 species. *Coxiella burnetii* was not detected, but it was possible to culture two new species of *Bartonella* from the blood, to detect *Leishmania infantum* by PCR and to identify rickettsia in the soft ticks parasiting these bats [25–27]. Recently, in cooperation with the veterinary services of New Caledonia, the bacterial "Candidatus Mycoplasma haemohominis" species was recently detected in fruit bats of the *Pteropus* genus. This bacterium is responsible for haemolytic fever in humans, an emerging zoonosis [28].

3.3. Rodents

Rodents represent nearly 40% of mammalian species. They are reservoirs and vectors of many zoonoses. They have been studied as part of the IHU's work in the Democratic Republic of Congo (DRC) as a reservoir for the plague [29], in Senegal, Mali and Afghanistan as reservoirs of *Bartonella* [30–32]. In the Côte d'Ivoire, it has been shown that the cane rat (*Thryonomys swinderianus)* can carry mycobacteria, precisely the *Mycobacterium ulcerans* which is responsible in humans for Buruli ulcer and zoonotic leptospira [33,34]. Numerous bartonellae and leptospirae have been found in rats from the city of Marseille [35–38]. Finally, hepatic capillariasis due to *Calodium hepaticum* has been identified in rodents from France Senegal and DRC [39,40].

3.4. Other mammalian species

A partnership concluded with two military hunting companies gave us access to wild boar (*Sus scrofa)* and red foxes (*Vulpes vulpes*) from the South-East of France. The hepatitis E virus genotype 3f in 2.5% (7/285) was thus detected by the PCR method in wild boar carcasses [41]. The seroprevalence of toxoplasmosis in these animals was 17% [42]. No tuberculous mycobacteria were detected in the faeces of these animals, but a new species of *Nocardia* was isolated [43]. Three zoonotic agents were detected in foxes: *Coxiella burnetii*, *Trichinella britovi* and *Leishmania infantum* [44–46]. For these last two agents, the fox acts as a reservoir. In addition, the haemoparasite *Hepatozooncanis* was detected in 92% of the 93 foxes tested [44]. This Apicomplexa is transmitted by ticks to canids. In French Guiana, our studies have shown that the three-toed sloth (*Bradypus tridactylus*) was a potential reservoir for *Coxiella burnetii,* the bacterium responsible for a serious human infection, Q fever, in this geographical area [47,48].

4. Screenings in domestic animals

4.1. Dogs

Dogs play a direct epidemiological role as a reservoir of pathogens [49]. They can also often act as asymptomatic sentinels for emerging infections [50]. The work carried out in the IHU on this species has focused on vector-borne diseases. This is the case for arboviruses, in particular the West Nile virus (WNV) infection and phlebovirus infections. Our studies have shown that the canine seroprevalence of the WNV is highly variable in different parts of Africa and Europe [51,52]. Military dogs, on their return from Africa, are good witnesses to the

Table 1
Zoonotic diseases the agents of which have been detected in reservoir (and/or sentinel) animals at, or in collaboration with, the IHU.

Diseases	Pathogens and detection techniques	Animals	Countries	References
West Nile fever	West Nile virus (S)	Dog	France (Corsica), Hungary, Djibouti, Chad, RD Congo, Cote d'Ivoire, Senegal	[51,52]
		Horse	France, Algeria, Senegal	[52,73–76]
		Goats	Senegal	[75]
		Donkey	Senegal	[75]
Hepatitis E	Hepatitis E virus (S, PCR)	Pig	France, DR Congo	[89,90]
	Hepatitis E virus (PCR)	Wild boar	France	[41]
Rift Valley fever	Rift valley fever virus (S)	Goats	Chad	[81]
		Sheep		
		Cattle		
Crimean-Congo hemorrhagic fever	Crimean-Congo hemorrhagic fever virus (S)	Horse	Senegal	[77]
		Cattle		
		Sheep		
		Goats		
		Donkey		
		Dog		
Toscana virus meningitis	Toscana virus (S)	Dog	France (Corsica), Algeria	[53,54]
Anaplasmosis	*Anaplasma phagocytophilum* (PCR)	Cattle	France, Algeria	[82,83]
Bartonellosis	*Bartonella* spp. (PCR)	Bat	French Guiana	[25]
	Bartonella rattimassiliensis (PCR, C)	Brown rat	France	[35,36]
	Bartonella phoceensis (PCR, C)	Brown rat	France	[35,36]
	Bartonella clarridgeiae (PCR, M, C)	Cat, Dog	France, Gabon	[69–71]
	Bartonella henselae (PCR, M, C)	Cat, Dog	France, Gabon	[69–71]
	Bartonella tribocorum (PCR) *Bartonella chomelii* (PCR, C)	Various rodents Cattle	Senegal France (New Caledonia)	[30,87]
Q fever	*Coxiella burnetii* (S, PCR)	Three-toed sloth	French Guiana	[47,48]
	Coxiella burnetii (S)	Dog	France	[55]
Lyme borreliosis	*Borrelia burgdorferi* (S)	Horse	France	[78]
West African tick-borne relapsing fever	*Borrelia crocidurae* (PCR)	Various rodents	Senegal	[30]
Leptospirosis	*Leptospira interrogans* serovar icterohaemorrhagiae (S, PCR)	Brown rat, Mice	France	[36–38]
	Several serovars of pathogenic leptospires (S)	Rusa deer	France (New Caledonia)	80
		Cattle	France (New Caledonia)	[80]
		Dog	France (New Caledonia), French Guiana, Gabon, Cote d'Ivoire, Senegal, Sudan	[56–58,80]
		Horse	France (New Caledonia)	[80]
Yaws	*Treponema pallidum* subsp. pertenue (S, PCR)	Green monkey, Baboon	Senegal	[16,17]
Hemolytic fever of megabats	*Mycoplasma haemohominis*	Bat	France (New Caledonia)	[28]
Buruli ulcer	*Mycobacterium ulcerans* (PCR, C)	Wild grasscutter	Cote d'Ivoire	[33]
Plague	*Yersinia pestis* (S)	Dog	DR Congo	[29]
Visceral leishmaniosis	*Leishmania infantum* (S, PCR)	Dog	France, French Guiana, Algeria, Cote d'Ivoire	[26,46,61,62]
	Leishmania infantum (PCR)	Fox	France	[44,46]
		Horse	Algeria	[61]
		Bat	French Guiana	[26]
	Leishmania infantum (S, PCR)	Red howler monkey	French Guiana	[19]
Cutaneous leishmaniosis	*Leishmania guyanensis* (PCR)	Red howler monkey	French Guiana	[19]
Toxoplasmosis	*Toxoplasma gondii* (S)	Wild boar	France	[42]
		Pig	France (New Caledonia), Cote d'Ivoire	[60,91]
		Dog	France (New Caledonia), Senegal, France	[59,60,91]
		Horse	France (New Caledonia), France	[60]
		Cattle	France (New Caledonia), France	[60]
		Cat	France (New Caledonia)	[60]
		Rusa deer	France (New Caledonia)	[60]
		Sheep	Senegal	[59]
		Goats	Senegal	[59]
		Donkey	Senegal	[59]
Chagas disease	*Trypanosoma cruzi* (S, PCR)	Dog	French Guiana	[63]
Hepatic capillariosis	*Calodium hepaticum* (AP)	Brown rat, other rodents	France	[39]
		Black rat	RD Congo	[40]
		Gambian pouched rat	Senegal	[40]
Dirofilariosis	*Dirofilaria immitis* (DO, S, PCR)	Dog	France, French Guiana, France (New Caledonia), Algeria, Cote d'Ivoire, Gabon, Sudan	[64–67]
	Dirofilaria repens (PCR)	Dog	France	[65]
Trichinellosis	*Trichinella britovi* (M, PCR)	Dog	Kosovo	[68]
		Fox	France	[45]

Abbrevations = DO: direct observation; S: serology; M: microscopy; AP: anatomic pathology; PCR: polymerase chain reaction; C: culture.

circulation of the virus in the ecosystems where they had been staying [49]. As for the Toscana virus, we have been able to identify seropositive dogs in Corsica and Algeria [53,54]. Canine seroprevalence surveys conducted in Africa, Corsica, French Guiana and New Caledonia have revealed the existence of risk areas for zoonoses, such as the plague [30], Q fever [54], leptospirosis [55–57] and toxoplasmosis [58,59]. Studies on canine leishmaniasis have been carried out in South-East France, but also in Algeria and, recently, the parasite *Leishmania infantum* has been detected (by positive PCR and sequencing) in autochthonous carrier dogs in French Guiana and Côte d'Ivoire [26,46,61,62]. In addition, for the first time, our work identified cases of asymptomatic infection by *Trypanosoma cruzi,* in dogs from French Guiana. This infection is the causal agent of Chagas disease in humans (screening of the parasite carried out by serology and PCR) [63]. Numerous studies have also been conducted on canine filariasis due to *Dirofilaria immitis* and *D. repens* [64–67]. These are zoonotic parasitoses the range of which is now on the increase in Europe. Finally, the presence of *Trichinella britovi* has been detected in the muscles of stray dogs in Kosovo and these represent sentinels, a potential risk for human infestation with this parasite [68].

4.2. Cats

IHU research on stray cat populations has led to the cultivation of two bacteria, *Bartonella henselae* and *B. clarridgeiae* [69,70]. In an original way, our work has contributed to the demonstration of the persistence of *Bartonellla* spp. in the pulp of teeth extracted from the skeletons of cats [71]. During an investigation on animals present in the home of two patients suffering from spotted fever due to *Rickettsia sibiricamongolotimonae*, it was shown that the ticks (*Rhipicephalus pusillus)* of the patients' cat were the reservoir and vectors of this rickettsiosis [72].

4.3. Horses

Horses and donkeys are sentinels of WNV circulating in a given ecosystem. Our work confirmed this in Corsica, in various African countries, in particular in Djibouti, North-West Senegal and Algeria [52,73–76]. Our work has made it possible to show, for the first time in Senegal, that horses were sentinels of the circulation of the Crimean-Congo haemorrhagic fever virus [77]. A seroprevalence survey of the Lyme disease conducted in France and Africa reported the absence of this disease south of the Sahara [78]. In France, our work contributed to the confirmatory diagnosis of the first described case of an equine infection with *Anaplasma phagocytophilum,* an agent of a rare zoonosis transmitted by ticks [79]. Finally, in New Caledonia, where leptospirosis is a public health problem, seroprevalence of this disease agent has been demonstrated, thus confirming the role of horses as sentinels of the infection [80]. In addition, leishmaniosis DNA was detected by PCR in the blood of a horse in Algeria [61].

4.4. Livestock

In cattle in Chad, seroprevalence surveys made it possible for us to highlight for the first time the circulation of an arbovirosis, namely Rift Valley Fever which is potentially serious in humans, [81]. In France and Algeria, clinical bovine infections due to *A. phagocytophilum* were diagnosed [82,83]. We have also shown the frequency of cattle infections with bartonelles in Africa [84–86]. In New Caledonia, six strains of bartonelles isolated from cattle were close to the reference strain of *Bartonella chomelii* and, in all probability, were imported from France along with cattle of the Limousin race [87]. In south-eastern France, an investigation was carried out demonstrating how ewes infected with *Coxiella burnetii,* the agent of Q fever, transmitted the infection to the owner of these animals [88]. Our studies have also highlighted the role of pigs as a reservoir for the hepatitis E virus [89,90] and toxoplasms [91]. Finally, in poultry, we have studied how the carriage of extended-spectrum beta-lactamases in faeces, are responsible for antibiotic resistance in human medicine [92].

5. Conclusion

The work carried out by the IHU on zoonotic agents and animal reservoirs has led to scientific discoveries and advances in epidemiological knowledge in this field. These results illustrate the need to continue this cross-cutting and integrated research approach (One Health) within the framework of the activities carried out within the IHU and, more generally, in the scientific programmes on zoonoses. Improving the identification of the agents of zoonoses and the assessment of the risk factors related to these agents will make it possible to implement better responses for the benefit of human and animal health and to contribute to a more conservation of biodiversity [93–95].

Authors' contributions

BD conceived the original paper and wrote the initial draft. SWG, CD, DR and OM extensively revised and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

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Declaration of Competing Interest

There are no competing interests.

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