4. Zoos and wildlife parks: a laboratory for the study of mosquito-borne wildlife diseases

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Abstract

Zoos and wildlife parks offer a variety of biotopes and habitats for all life stages of numerous mosquito species and are places of close encounters between mosquitoes, captive animals, wild native animals and human visitors. Since stock animals of modern zoos/wildlife parks are closely observed by their keepers and medically attended by veterinarians, disease agents transmitted to them by mosquitoes will come to attention as soon as they cause symptoms or the animals are routinely checked. In addition to giving medical care to the affected animal, zoo/wildlife park staff has then the possibility to induce epidemiological investigations. The latter should be done in cooperation with the higher veterinary authority or, in case of a zoonotic disease agent, with the responsible public health authority, or with both. Zoos/wildlife parks can thus be valuable sentinel stations for detecting local circulation and transmission of mosquito-borne disease agents and significantly contribute to public health surveillance. Sometimes, zoos/wildlife parks also offer the opportunity to study mosquito-borne infections or disease cases in susceptible non-natural hosts or in natural hosts kept under non-natural conditions or exhibiting non-natural behaviour. These situations allow important insights into disease etiology and pathology as well as into vector biology. With respect to the 'One Health' approach, zoos, wildlife parks and similar facilities, where exotic and non-adapted animals are exposed to locally occurring (native or imported) vector-borne pathogens, should gain much more attention regarding pathogen surveillance and public health issues.

Keywords: animal health, biosurveillance, pathogen transmission, public health, sentinel, vector

Introduction

Detecting rare, including emerging, endemic and zoonotic vector-borne pathogens in the field can be a search for the needle in the haystack. However, the knowledge on the presence and circulation of such pathogens in a given area will help raising awareness and facilitate or direct the design and implementation of measures to prevent or manage disease cases among animals and humans (Braks *et al.* 2011). Accordingly, disease and pathogen surveillance and research in wildlife is a major issue of the One Health approach (Sleeman *et al.* 2019).

For reasons of resources, vector-borne pathogen surveillance is rarely done comprehensively and proactively. It usually only occurs in response to noticeable events such as the emergence of local disease cases or infections, or the establishment of a new vector species. Surveillance may be performed on potential vectors with acquisition of these to be screened for pathogens (xenomonitoring) usually taking place by random trapping, and less often by collecting from human or animal baits (Silver 2008). Much more elaborate and delicate is the examination of animal hosts for infection, as these have to be arduously captured and sampled by invasive

techniques. Moreover, such activities usually underly protection of animal acts and have to be formally permitted (Soulsbury *et al.* 2020). Both vector and vertebrate samples can be examined for the pathogens by microscopy (histology of body fluids or tissue samples), culturing (of body fluids) or biochemical and genetic methods (e.g. MALDI-TOF, PCR assays) (Reller *et al.* 2017). Additionally, vertebrates can be indirectly tested for infection by serological techniques (Kuleš *et al.* 2017) and, in some cases, directly by xenodiagnosis (e.g. Meiser and Schaub 2011, Mourya *et al.* 2007, Turk *et al.* 2018). However, large-scale programmes on vector-borne pathogen surveillance are rarely in place, and pertinent activities are often only performed in the framework of research projects.

A special situation exists in zoos and wildlife parks, where animals are generally under thorough observation and, in most cases, routinely and regularly examined for their health status (Carpenter *et al.* 2016). Certainly, this is particularly important for animals taken from the field or originating from unknown or dubious sources to supplement the animal stock in a zoo/wildlife park. In addition to being medically examined, they often have to pass a quarantine period before being released to their conspecifics in order not to introduce any external disease agents (Carpenter *et al.* 2016). However, nowadays animals are rarely taken from the field to be held captive in zoos. Most of them result from breeding programmes that aim to conserve species at risk of extinction or increase the genetic diversity of animals and therefore have already been born and bred in captivity, hence have been medically attended since birth (IUCN 2014, Mallinson 1995). Based on this, it can be assumed that animals are healthy when they enter a zoo and may acquire an infection only in the present zoo setting. This implies that vector-borne pathogens detected in zoo animals are generally not exotic but have a local source.

Haematophagous ectoparasites that may serve as vectors in zoos or wildlife parks are rarely stationary and permanent, since these have little chance of being introduced. Due to the impact they have on hosts, medical checks are routinely performed on introduced vertebrates, with their high detection capacity leading to the quick detection and elimination of the ectoparasites by pest control activities. In addition, stationary and permanent vectors have limited occasions to circulate pathogens, unless between vertebrates of the same flock or in the same enclosure. Vector-borne pathogens are therefore mainly introduced by mobile arthropod species difficult to control, with mosquitoes playing a crucial role (Adler *et al.* 2011).

Mosquitoes in zoos/wildlife parks

Mosquitoes as potential vectors of pathogens find excellent developmental and living conditions in zoos and wildlife parks (Heym *et al.* 2018). First, these provide numerous and different kinds of breeding sites (Tuten 2011). Zoos and wildlife parks usually contain a lot of small and medium-sized water places of any kind in order to offer appropriate and pleasant settings for both captive animals and visitors. Containers used as drinking troughs are probably unproblematic as mosquito production sources since the water is replaced every few days at least, not permitting aquatic mosquito stages to complete their development. However, other man-made constructions, be they ornamental or necessarily linked to buildings and animal shelters, such as rainwater gutters, gullies and cesspits, may collect water and serve as persistent mosquito breeding habitats. Second, zoos and wildlife parks are usually heavily vegetated providing shadowy places where adult mosquitoes rest and plenty of sources to acquire nectar and sweet plant juices. Finally, potential blood hosts are available in considerable numbers: captive vertebrates, free-roaming native birds, rodents and other small vertebrates, and human visitors (Vezzani 2007).

Mosquito eggs, larvae and pupae can be imported into zoos/wildlife parks by plants and gardening equipment, while adults may enter from outside, e.g. urban or forested areas, depending on their flight radius. For example, the average flying distances of *Culex pipiens* and *Aedes vexans*, two of the most common and widely distributed mosquito species in Europe, are 400-500 hundred and a good hundred metres, respectively (Verdonschot and Besse-Lototskaya 2014).

Thus, mosquitoes are omnipresent in zoos and might easily transmit pathogens from wild native animals to captive animals and between visitors and zoo animals.

Mosquito-borne infections in zoos/wildlife parks

Except for avian malaria, publications on mosquito-borne infections in zoos and wildlife parks are few, suggesting either a limited number of cases or underreporting in the scientific literature. As the task of zoo and wildlife park staffs has no scientific priority, the latter is more likely. Of the pertinent literature published, most articles pertain to infections with viruses and avian malaria parasites.

Viral infections

Several reports exist on mosquito-borne viral disease incidents in vertebrates or on mosquitoes infected with viruses, in particular West Nile virus (WNV), from zoos and wildlife parks. While published studies on infections in vertebrates mainly refer to seroprevalences, those on mosquitoes predominantly describe genetic pathogen detection approaches.

Expectedly, anti-WNV antibodies cannot only be found in vertebrates believed to be susceptible to WNV infections (i.e. may develop disease symptoms), such as certain groups of birds and horses, but in others having come into contact with infectious mosquitoes as well, e.g various mammal or reptile species (see below). However, also symptomatic WNV infections were observed in exotic animal species. Zoo birds fell ill or were tested seropositive in considerable numbers, e.g. in Kansas and New York City (USA), Córdoba (Spain) and Tabasco (Mexico) (Cano-Terriza *et al.* 2015, D'Agostino and Isaza 2004, Hidalgo-Martínez *et al.* 2008, Ludwig *et al.* 2002). In the Bronx Zoo, New York, captive vertebrates were found seropositive for infection during the 1999 WNV outbreak, with 34% of tested birds and 8% of tested mammals positive (Ludwig *et al.* 2002). Comparison with stored sera provided no evidence for WNV infection before 1999 and demonstrated that the virus did not originate from a captive bird introduced into the zoo stock.

In one zoo in Tabasco (Mexico) about a third, each, of all tested birds (numerous species) and mammals (several species including antelope and big cats) were seropositive, while six of seven tested crocodiles (86%) had anti-WNV bodies. One pool of *Culex quinquefasciatus* was found WNV-positive by PCR (Hidalgo-Martínez *et al.* 2008).

Most remarkable were the cases of symptomatic WNV infections in a polar bear in the Toronto Zoo in Canada in 2006, and in an orca in the SeaWorld park in Texas, USA, in 2007. The bear was euthanised after displaying paraparesis and a poor general condition. It was tested seropositive for WNV after symptoms had been recognised, and WNV could be cultured and detected by PCR in spleen samples taken post mortem (Dutton *et al.* 2009). Infection in orcas is particularly noteworthy as these would probably hardly become infected in nature where exposition to mosquitoes is difficult to imagine: in their natural marine habitat, orcas are mostly not close enough to the shore and do not spend enough time at the water surface for mosquitoes to bite. In addition,

mosquitoes keep away from turbulent waters as these pose a threat to their life. Notwithstanding, infections with WNV as well as with eastern, western and Venezuelan encephalitis viruses have serologically been shown to occur in free-ranging Atlantic bottlenose dolphins, although no disease symptoms were registered (Schaefer *et al.* 2009). The orca in the Texas SeaWorld died suddenly without obvious signs of illness but autopsy showed lesions of the central nervous system typical for WNV infection. Also, WNV was genetically confirmed in brain tissue (St. Leger *et al.* 2011).

In captivity, orcas spend most of their time just floating motionless at the smooth water surface of their pools, in a surrounding where mosquitoes may easily approach and feed (Jett and Ventre 2012). Jett and Ventre (2012) see further risk factors of orcas to suffer from mosquito-borne viral diseases in their generally poor health condition in captivity, resulting from (1) higher UV radiation and epidermal lesions following sunburn due to a lot of time spent at the water surface in marine parks in the southern USA as compared to their natural occurrence at more northern latitudes, and (2) ill dentition due to biting concrete and metal structures in their spatially limited pools.

After the repeated occurrence of WNV cases among captive birds in the Berlin Wildlife Park in 2018 and 2019 (Ziegler *et al.* 2019, 2020), Kampen *et al.* (2020) screened mosquitoes collected in the park in autumn 2019 and found seven WNV-positive *Cx. pipiens* complex pools including the two biotypes *pipiens* and *molestus*.

WNV is also a health threat to humans and horses, although being dead-end hosts for the virus (Chancey *et al.* 2015). Most infections remain undetected and pass by without any symptoms but some 20% of the infected individuals develop unspecific flu-like symptoms. While most of these quickly recover, the virus becomes neuroinvasive in 1% of the patients and may lead to severe impairment of the central nervous system including death (Rossi *et al.* 2010).

A similar situation is given with St. Louis encephalitis virus (SLEV) which is believed to have killed an orca already in 1990 in the Florida SeaWorld (Buck *et al.* 1993): most patients will recover spontaneously, some will display mild symptoms and only few, mainly elder ones, will develop encephalitis. For the latter, the case fatality rate can reach 20% (Reisen 2003). The orca in Florida died after presenting with reduced appetite and lethargy. Diagnosis was based on neutralisation tests and immunofluorescence staining, supported by virus-induced cytopathic effect in inoculated cell cultures and electron microscopy of culture cells (Buck *et al.* 1993).

Neutralising antibodies against SLEV, as well as against Zika virus (ZIKV) and dengue virus (DENV) serotypes 2, 3 and 4, were also found in capuchin monkeys in the Recife Zoo, Pernambuco, Brazil (De Oliveira-Filho *et al.* 2018). Both ZIKV and DENV can lead to severe disease symptoms in humans. ZIKV gained global attention during the large epidemic in South America in 2015 and 2016, with numerous children born with microcephaly (Hills *et al.* 2017). In adults, ZIKV infection may be associated with Guillain-Barré syndrome (Silva *et al.* 2020). DENV infection may either remain asymptomatic or have a mild or severe disease course. The mild outcome is characterised by flu-like symptoms, while the severe one may include dengue hemorrhagic fever and dengue shock syndrome. The risk of developing severe dengue increases with a second infection of a different virus serotype (Silva *et al.* 2020).

In Connecticut, USA, 14 African penguins (*Spheniscus demersus*) out of a flock of 22 held at a public aquarium were diagnosed with eastern equine encephalitis (EEE) in 2003 (Tuttle *et al.* 2005). This was the first time, clinical signs of EEE virus (EEEV) infection had been described in penguins.

Diagnosis was based on hemagglutinin inhibition and virus neutralisation tests after development of substantial neurological signs in 13 of the penguins. The same number of penguins survived due to intensive supportive care while one juvenile penguin had to be euthanised since clinical symptoms did not improve. Although very rare in canines, too, two Mexican gray wolf pups died of EEEV infection in Binder Zoo Park, Michigan, USA, in 2019 (Binder Park Zoo 2019). EEEV is a primary virus of horses. In humans, flu-like symptoms may occur, but severe disease cases have been recorded with fatality rates of up to 35% (Kumar *et al.* 2018).

Usutu virus (USUV) has become a common and widespread mosquito-borne virus in Europe. It emerged in Austria in the early 2000s (Weissenböck *et al.* 2002), although it was later shown to have been present in Italy already in 1996 (Weissenböck *et al.* 2013). Since that time, avian USUV infection and mortality were recorded across many European countries (Vilibic-Cavlek *et al.* 2020), with several cases described from zoological gardens or private aviaries in Switzerland, Germany, Austria, Spain and the Netherlands (Becker *et al.* 2012, Buchebner *et al.* 2013, Cano-Terriza *et al.* 2015, Rijks *et al.* 2016, Steinmetz *et al.* 2011, Ziegler *et al.* 2016). USUV is a virus of birds, and its zoonotic potential is still under discussion. Few symptomatic cases have to date been reported in humans, mainly splenectomised patients (Cavrini *et al.* 2009, Pecorari *et al.* 2009). However, rare neuroinvasive cases have also been described from people without any pre-existing adverse conditions (Nagy *et al.* 2019, Simonin *et al.* 2018, Vilibic-Cavlek *et al.* 2014).

Sindbis virus (SINV), mainly transmitted by *Cx. pipiens, Culex torrentium, Aedes cinereus* and *Aedes excrucians* (Lundström 1994), can cause sporadic illness that is rarely connected to a fatal outcome in birds and a flu-like disease in humans which sometimes leads to chronic arthritis (Hubálek 2008). This virus was detected in a pool consisting of *Cx. pipiens* complex mosquitoes collected in the Berlin Wildlife Park (Heym *et al.* 2019).

Protozoan infections

Compared to other vector-borne infections in zoos and wildlife parks, a relatively large body of literature exists on cases and outbreaks of avian malaria and malaria-like disease which are caused by protozoan parasites of the genera *Plasmodium*, *Leucocytozoon* and *Haemoproteus*. Only species of the genus *Plasmodium*, which are ubiquitous and common, are believed to be transmitted by mosquitoes, while *Leucocytozoon* parasites are thought to be mainly transmitted by black flies (Simuliidae) and *Haemoproteus* by biting midges (Ceratopogonidae) and keds (Hippoboscidae), respectively (Service 2001). While these parasites generally do not do appreciable harm to co-evolved and adapted birds, they may be life-threatening to, and cause mass mortality in, bird species not adapted to the infectious agent (Bennett *et al.* 1993, Valkiūnas 2005). In the early 19th century, the *Cx. quinquefasciatus* mosquito was introduced into Hawaii by humans. This species, a competent vector of avian plasmodial protozoans, enabled the transmission of *Plasmodium relictum* from introduced to native birds and almost caused the extinction of Hawaiian honeycreepers (a passerine subfamily) due to avian malaria (Van Riper *et al.* 1986, Warner 1968). A high diversity of genetic lineages of avian malaria parasites suggests the existence of numerous undescribed species (Valkiūnas and Iezhova 2018).

In zoos, penguins are particularly susceptible to avian malaria parasites, and disease outbreaks have been documented from all over the world (e.g. Baltimore Zoo, USA: Stoskopf and Beier 1979; Farmland Zoo, Korea: Bak and Park 1984; São Paulo Zoo, Brazil: Bueno *et al.* 2010; Hai Park Zoo, Israel: Lublin *et al.* 2018). Certain species of penguins present particular high sensitivities to this parasites, probably caused by the fact that they have little or no contact to mosquitoes and

mosquito-borne pathogens in their natural surroundings, resulting in a naive immune system (Jones and Shellam 1999, Jovani *et al*. 2001). The high mortality rates of up to 50-80% in captivity due to avian malaria have prompted many zoos to systematically test their penguins for plasmodia and to administer antimalarial drugs prophylactically (Grilo *et al*. 2016).

However, avian malaria infections caused by plasmodia have also been reported from captive bird species other than penguins, e.g. pheasants (Murata *et al*. 2008), yellowheads (Alley *et al*. 2008), Egyptian goose (Fernandes-Chagas *et al*. 2013), flamingos (Thurber *et al*. 2014) and various species of cranes (Jia *et al*. 2018).

As opposed to the considerable number of articles on avian malaria in zoos and bird holdings, hardly any studies have been published on mosquito vectors in these settings. In a zoological garden in Kanagawa (Japan) Eijiri *et al*. (2009) found plasmodial DNA in *Lutzia vorax* and *Cx. pipiens* group mosquitoes which they specified as *Cx. pipiens pallens* in a subsequent study (Eijiri *et al*. 2011). Heym *et al*. (2019) demonstrated the DNA of *Plasmodium* sp. in *Cx. pipiens* biotype *pipiens* as well as *Haematoproteus*- and *Leucocytozoon*-DNA in undefined specimens of the *Cx. pipiens* complex in the Berlin Wildlife Park and the Eberswalde Zoo, Germany. In the Barcelona Zoo (Spain), Martínez-de la Puente *et al*. (2020) found DNA of various *Plasmodium* strains in *Cx. pipiens* s.l. and of a *Haemoproteus* strain in *Culiseta longiareolata*. In Bioparco Zoological Garden and Zoomarine Park in the Lazio region, Central Italy, Iurescia *et al*. (2021) identified *Plasmodium matutinum*-DNA in both deceased African black-footed penguins and in *Cx. pipiens* s.l., and *Plasmodium vaughani*-DNA in *Cx. pipiens* s.l. only. In the São Paulo Zoo, Brazil, De Oliveira-Guimarães *et al*. (2021) demonstrated *Plasmodium nucleophilum*-DNA in *Aedes albopictus*, *Aedes scapularus* and *Culex acharistus* and *Mansonia indubitans*, in addition to non-specified plasmodial DNA in indeterminable *Culex* species. Interestingly, *Haemoproteus*-DNA was found in head/thorax samples of an *Ae. scapularis* female and three *Mansonia* sp. females, suggesting presence of metacyclic stages of this haemosporidial genus, which is considered to be non-culicid-borne, in the mosquitoes' salivary glands. At Aquarium Marinepoia Nihonkai, Niigata, Japan, Inumaru *et al*. (2021) identified two *Plasmodium* lineages in both mosquitoes fed on penguins (*Cx. pipiens* group, *Culex tritaeniorhynchus*) and in penguins, whereas *Plasmodium cathemerium* was only detected in mosquitoes, suggesting that captive and wild birds have their own specific roles in avian malaria.

Filarial infections

Filarial worms may produce disease in all groups of vertebrates, and several species are zoonotic. For example, culicids transmit species of the genera *Dirofilaria* (pathogenic to carnivores and humans), *Setaria* (pathogenic to ruminants and equids) and *Cardiofilaria* (pathogenic to birds) (Mehlhorn 2016).

The heartworm *Dirofilaria immitis* is a parasite of caniforms, transmitted by mosquito species of various genera. In addition to humans, it can occasionally be found in feliforms, such as in the case of a snow leopard in the Kobe Municipal Oji Zoo, Japan (Murata *et al*. 2003). The leopard died of pancreatic carcinoma, and the filariae were incidentally detected in the right cardiac ventriculus during necropsy. During the leopard's lifetime, they had not caused any obvious clinical signs. More extraordinary is the symptomatic infection of a Humboldt penguin by *D. immitis* in Akita Municipal Omoriyama Zoo, Japan, assumed to be causative for the death of the penguin (Sano *et al*. 2005). The filariae were found post mortem in the right atrium of the heart and in the connective tissue of the lung. In both, leopard and penguin, filarial species diagnosis was carried out using morphological and genetic tools.

Contribution of zoos/wildlife parks to mosquito-borne disease research

Mosquito ecology

Ecological and behavioural characteristics of mosquito vectors contribute to presence and abundance as well as pathogen transmission. Zoos and wildlife parks offer excellent surroundings to analyse mosquito blood meals that provide conclusions about host preferences and feeding behaviour (Heym *et al.* 2018, Martínez-de la Puente *et al.* 2020, Schönenberger *et al.* 2016, Tuten *et al.* 2012). The identification of breeding and resting sites of specific mosquito species gives valuable information for exposure reduction in zoos or even zoo construction. Furthermore, flight distances can be accurately assessed for blood-fed mosquitoes by measuring distances between their collection sites and the enclosure of the vertebrate species fed upon (Greenberg *et al.* 2012, Heym *et al.* 2018, Martínez-de la Puente *et al.* 2020, Schönenberger *et al.* 2016, Tuten *et al.* 2012). *Ae. vexans*, for example, was captured at an average distance of 109.2 m (18 m minimum, 167 m maximum) away from its blood host in the Rio Grande Zoo, Albuquerque, New Mexico (Greenberg *et al.* 2012), while *Cx. pipiens* s.l. exhibited mean post-blood meal flight distances between 62 and 236 m, depending on the gonotrophic conditions (Sella stage), in Greenville and Riverbanks zoos, South Carolina (Tuten *et al.* 2012) and a mean post-blood feeding flight distance of 99 m (maximum 168.5 m) in Barcelona Zoo, Spain (Martínez-de la Puente *et al.* 2020).

In addition to blood of various zoo animal species found in the mosquitoes, human blood was detected in *Ae. albopictus, Aedes annulipes* s.l., *Ae. cinereus, Aedes japonicus, Aedes punctipennis, Ae. vexans, Anopheles claviger, Anopheles maculipennis* s.l., *Anopheles plumbeus, Culex erraticus, Culex hortensis* and *Cx. pipiens* s.l. (Greenberg *et al.* 2012, Heym *et al.* 2018, Martínez-de la Puente *et al.* 2020, Schönenberger *et al.* 2016, Tuten *et al.* 2012).

Deeper knowledge of all these aspects facilitates the preparation of risk assessments for both zoo animals and human citizens, as well as the design and implementation of targeted vector and disease management. A closer collaboration between the animal and public health sectors is therefore most desirable.

Biosurveillance

As routine surveillance of mosquito-borne pathogens in the field is exceptional, zoos and wildlife parks play a paramount role as epidemiological monitoring stations and significantly contribute to the detection of zoonoses and emerging infectious diseases. Domesticated and captive animals are kept close to humans, sharing the same or similar environments, but spend much more time outdoors, thereby displaying an increased exposure risk (Rabinowitz *et al.* 2013). Thus, the routine work of zoos and wildlife parks on attendance and treating of animals in the interest of conservation also significantly serves the public health sector (McNamara 2007). It can be assumed that infections in zoo animals will be detected relatively soon, either during routine examinations or after the onset of symptoms, and that they will be taken care of and followed up for their etiology. This includes re-examination of test material taken previously from the same animals which are commonly kept banked for some time. The animals are frequently observed and monitored, and in the case of death undergo complete necropsy and any additional disease testing to determine the cause of death (Binder Park Zoo 2020).

The contributions zoological institutions are able to make for public health and their relevance have been identified and analysed retrospectively for the case of WNV in the US (Pultorak *et al.*

2011). During the 1999 outbreak in New York, examinations of fresh and archived animal samples from the Bronx Zoo helped unveil the etiology and epidemiology of mysterious avian morbidity and mortality, identifying the cause as WNV infections (Ludwig *et al.* 2002). To this end, animals represent important sentinels for the surveillance of human health hazards in general (Neo and Tan 2017), with a particular role of zoo/wildlife park animals, in addition to exhibiting (exotic) biodiversity and serving for conservation purposes. Moreover, captive animals rarely live under natural conditions in zoos and wildlife parks, i.e. in different geographical areas and/or forced to display non-natural behaviour, and might be exposed to pathogens not occurring in their native distribution area and natural habitats. Hence, combinations of pathogen and host may turn up not found in nature. This may be detrimental to the individual vertebrate affected, but may significantly add to our knowledge on pathogen-host interactions, vector ecology, and disease pathology and epidemiology. In this context, it is to be kept in mind that haematophagous arthropods in which pathogens are detected by genetic techniques may not necessarily be vectors of the demonstrated pathogens. Most often, the whole bodies of arthropods are homogenised for examination, not differentiating between crucial body parts such as midgut and head/salivary glands. Only demonstrated experimental transmission or the detection of infectious pathogens in the salivary glands (viruses, protozoa) or head (filarial worms) after natural infection qualifies a mosquito to be called a biological vector.

Climate change

Zoos and wildlife parks can also contribute to infectious disease research with respect to climate change as they may have possibilities to simulate different climatic conditions and have available long-term historical data on animal health (Barbosa 2009). Of 118 transmissible diseases affecting animals in zoos and aquariums (Kaandorp 2004), 29, including several vector-borne ones, are considered likely to be impacted by climate change (Barbosa 2009). Therefore, under the auspices of the World Association of Zoos and Aquariums (WAZA), project MOSI was established, a zoo-based mosquito monitoring network aimed at assisting wildlife health management and contributing data on mosquito spatio-temporal distribution changes (Quintavalle Pastorino *et al.* 2015). In this programme, composition, abundance and acitivity profiles of mosquito species and their changes over time are to be determined in various European zoos. Moreover, mosquitoes will be examined for blood meal sources and pathogen presence. Assistance in risk evaluation and management of mosquito-transmitted pathogens in the zoo environment will be provided.

Concluding remarks and future directions

Although several mosquito species collected in zoos and wildlife parks have been found infected with mosquito-borne disease agents pathogenic to animals held captive, knowledge on their vector competence, vector capacity and actual vector role in these particular settings is scarce. Prevalence studies on wild animals foraging within the zoos or within flight distance of mosquito species occurring in zoos that may present infection sources, such as birds, might help better assess the infection risk for zoo animals and design prevention strategies. Measures to control mosquitoes and reduce exposition of zoo animals are mainly sanitary, i.e. consist of elimination, reduction and proper management of potential mosquito breeding places, be they natural or artificial (Derraik 2005). The regular application of larvicides (e.g. *Bacillus thuringiensis israelensis* (Bti) and *Bacillus sphaericus* toxins; Lacey 2007) to water bodies in and around the zoos and the use of insect meshes on cages with particularly endangered species and facility windows is worth considering. However, monitoring of both zoo/wildlife park animals and mosquitoes for

pathogens is a most efficient early warning tool that allows to take targeted action in due time to prevent or reduce mosquito-borne disease agent transmission to both zoo animals and humans.

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