Viral discovery in captive polar bears (*Ursus maritimus*)

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Zusammenfassung

Zwei gemeinsam im Zoologischen Garten Wuppertal untergebrachte Eisbären (*Ursus maritimus*) erlitten im Jahr 2010 epileptiforme Anfälle, woraufhin der weibliche Eisbär „Jerka“ acht Tage später verstarb. Der männliche Eisbär „Lars“ überlebte nach einer symptomatischen tierärztlichen Behandlung. „Knut“, der Sohn von „Lars“, welcher im Zoologischen Garten Berlin lebte, erlitt ebenfalls epileptiforme Anfälle und ertrank in deren Verlauf im Jahr 2011. Die zwei Todesfälle, welche unter ähnlichen Umständen eintraten, führten in der zoologischen Gesellschaft und der Öffentlichkeit zu der Befürchtung, dass ein Infektionserreger diese charismatische Tierart bedrohen könnte. Das Ziel dieser Arbeit ist es, Methoden zur Identifikation eines Erregers bei in Gefangenschaft und frei lebenden Tieren zu entwickeln, und diese bei der Aufklärung der Todesursache beider Eisbären anzuwenden.

Kapitel II beschreibt detailliert ein dreistufiges Diagnoseverfahren, inklusive der Einschränkungen und Vorteile jeder Methode. Das Verfahren wird angewendet um die Ursachen des Todes von „Jerka“ und „Knut“ festzustellen. Im ersten Schritt des Verfahrens wird eine Sektion, sowie eine histologische und bakteriologische Untersuchung durchgeführt, um potenzielle Pathogene auszuschließen, bzw. Erreger-Kandidaten zu bestimmen. Die Sektion ergab, dass beide Eisbären Anzeichen einer nicht-eitrigen Panmeningoenzephalitis zeigen höchst wahrscheinlich aufgrund viraler Genese. DNA- und RNA-Extrakte aus Gehirn und Leber beider Tiere werden molekularen Diagnosetechniken wie der PCR, modernsten Virus Microarrays, Immunhistochemie und Next Generation Sequencing unterzogen. Der dritte Schritt des Verfahrens beinhaltet serologische Methoden wie den Serumneutralisationstest (SNT), den Hämagglutinationshemmtest (HAH-Test) und den ELISA. Die Ergebnisse der auf die Eisbären angewendeten Verfahren sind für „Jerka“ detailliert in Kapitel III und für „Knut“ detailliert in Kapitel IV beschrieben.

In Kapitel III dieser Arbeit untersuche ich die Ätiologie von „Jerkas“ Tod und „Lars“ Symptomen mit Hilfe molekularer Methoden. Die Sektionsergebnisse von „Jerka“ weisen darauf hin, dass das Tier an einer nicht-eitrigen Meningoenzephalitis, höchst wahrscheinlich viralen Ursprungs, verendete. Es werden PCR Untersuchungen und virale Mikroarrays mit den DNA- und RNA-Extrakten aus „Jerkas“ Gehirn durchgeführt, welche einen Großteil der Viren abdecken, die bei Tieren eine Enzephalitis verursachen.

Alle geprüften Pathogene sind negativ, außer das Equine Herpesvirus 1 (EHV-1). Das Vorhandensein des EHV-1 wird durch einen Western Blot bestätigt. Die genaue Analyse der sequenzierten Virusgene zeigt, dass das identifizierte Virus ein rekombinanter Virus aus dem Zebra-EHV-1 und EHV-9, einem anderen Zebra Herpes Virus ist. Es wurde bereits gezeigt, dass das EHV-9 andere Wildtierarten infiziert und zum Tod führt. Auch der Speichel von Lars war schwach positiv mittels qPCR. Diese Ergebnisse widerlegen das Dogma, das Herpesviren wirtsspezifisch seien und dokumentieren eine neue über Kreuz Transmission von Zebra-EHV auf Eisbären, ein Ereignis, welches sich in freier Wildbahn nie ereignen würde.

In **Kapitel IV** dieser Arbeit beschreibe ich detailliert den ersten Nachweis eines endogenen Retrovirus von Eisbär und großen Panda, welcher durch das in Kapitel II beschriebene Verfahren möglich wurde. Die Transkriptomanalyse der beiden Eisbären, „Knut“ und „Jerka“, zeigt eine retrovirale Sequenz, welche überraschenderweise der Gruppe der humanen endogenen Retroviren K HERV-K, ähnlich ist. Das Genomscreening der Eisbären ergibt 26 provirale Sequenzen des identifizierten endogenen Retrovirus welches UmaERV benannt wird. Die Datierung der extrahierten Proviren weist auf die Integration der Virusgruppe in das Genom vor 45 Millionen Jahren hin, das bedeutet die Invasion des Genoms durch das Virus fand statt, bevor der Bär sich von den Flossenfüßern abspaltete. Die Sondierung des Genoms des großen Panda (*Ailuropoda melanoleuca*) unterstützt dieses Ergebnis, da 20, dem UmaERV ähnliche, provirale Sequenzen gefunden werden. Das endogene Retrovirus der Pandabären wird AmeERV benannt. Um zu zeigen, dass es sich nicht um ein generelles Carnivorenretrovirus handelt, wird anhand eines Hunde- und eines Katzengenoms gezeigt, dass das Screening bei diesen Spezies negativ ausfällt. Die phylogenetische Analyse der Proteinkonsensussequenz der zwei Viren ergab eine Einordnung in die Gruppe der HERV-K, was zeigt, dass diese Virusgruppe deutlich komplexer ist, als angenommen.

Summary

In 2010, two co-housed polar bears (*Ursus maritimus*) in the Wuppertal Zoological Garden exhibited seizures that led to the death of the female polar bear Jerka, 8 days later. The male polar bear Lars, survived after receiving medical treatment. Knut, the son of Lars, of the Berlin Zoological Garden also suffered seizures and drowned in 2011. The two polar bear deaths with similar symptoms raised concerns among zoos and the public that infectious pathogens may be threatening a marquee and charismatic species. The aim of this thesis was to establish methods for the identification of causative agents of disease in captive and free living wildlife and explore their use in elucidating the cause of death in the polar bear cases described.

In **Chapter II** a three-stage diagnostic pipeline is described in detail demonstrating the limitations and advantage of each method used. The pipeline was used in an attempt to determine the causes of death of Knut and Jerka. In the first stage of the pipeline, necropsy, histology and bacteriology were used to include or exclude or suggest causative pathogens. Necropsy suggested that both bear cases represented viral encephalitis. Molecular diagnostics that included PCR, state of the art viral microarrays, immunohistochemistry, and next generation sequencing where performed on DNA and RNA extracts from both brain and liver from the two animals. The third step of the pipeline included serological methods like serum neutralization test (SNT), haemaglutination assay (HI), and ELISA. Results derived from the pipeline are detailed in chapter III for Jerka and chapter IV for Knut.

In **Chapter III** of this thesis I investigate the etiology behind Jerkas death and Lars symptoms using molecular techniques. The necropsy results of Jerka indicated that the animal suffered from non-suppurative encephalitis, most likely of viral origin. PCRs and viral microarrays were performed on DNA/RNA extractions from Jerka's brain covering the majority of viruses that were able to cause encephalitis in animals. All pathogens screened were negative except equine herpes virus 1 (EHV) result that was verified by Western blot assay. More detail analysis of the viral genes sequenced revealed that the virus identified was a recombinant zebra EHV1 with EHV9 another zebra herpes virus that had previously showed to infect and cause death to other wild species. Lars' saliva was also weakly positive. The results violate the dogma

that herpesviruses are host specific and document a novel cross species transmission of EHV to polar bears from zebras, an event that could never happen in the wild.

In **Chapter IV** of this thesis I describe in details the first polar bear and giant panda endogenous retrovirus that was identified through the use of chapter II described pipeline. Transcriptome analysis of the two polar bears, Knut and Jerka, revealed a retroviral sequence surprisingly similar to the human endogenous retrovirus (HERV) K group, HERV-K. Polar bear genome screening revealed 26-proviral sequence of the identified endogenous retrovirus that was named UmaERV. Dating of the extracted proviruses suggested this group integrated genomically 45 million years ago indicating that the genomic invasion of this retrovirus occurred before the split of the bear from the pinniped lineage. Screening of the giant panda (*Ailuropoda melanoleuca)* genome verified the above result as 20-proviral sequences similar to the UmaERV were identified. The giant panda endogenous retrovirus was named AmeERV. In order to verify that this is not a general carnivore retrovirus the dog and cat genome were screened, and were negative. Phylogenetic analysis of the protein consensus of the two viruses placed them within the human endogenous retrovirus K group illustrating that the evolutionary history of this group of viruses is far more complex than previously thought.

Chapter I
General Introduction

General Introduction

1.1 Relationships among the Ursidae

The *Ursidae*, a family of mammals that belongs to the Carnivora group, is comprised of eight living species subdivided into three genera: Ailuropoda, which includes the giant panda (*Ailuropoda melanoleuca*) and the only living non-carnivorous bear, Tremarctos with only one living member, the spectacled bear (*Tremarctos ornatus*), and Ursus which is comprised of brown (*Ursus arctos*), polar (*Ursus maritimus*), sloth (*Melursus ursinus*), sun (*Helarctos malayanus*), Asiatic (*Ursus thibetanus*) and American black (*Ursus americanus*) bears. All bear genera are classified as endangered at the species or subspecies level and many have very high conservation status (Yu et al. 2007). Bears provide evolutionary models of complex speciation and rapid evolution while also presenting a challenging taxonomy with controversial phylogenetic relationships among its members (Yu et al. 2004, Miller et al. 2012). Mitochondrial DNA and nuclear gene phylogenetic analysis of the Ursidae indicates that the giant panda lineage is basal to other bear lineages with the spectacle bear representing the first genus to branch subsequently (Figure 1). This divergence occurred in the mid Miocene approximately 20 mya (Yu et al. 2004, Hailer et al. 2012, Miller et al. 2012). The sloth and sun bears represent the sister taxons to the Ursids (Figure 1) (Yu et al. 2007). The polar bear - brown bear divergence is the most recent speciation event among the Ursidae having occurred between 2 mya - 600 kya. (Yu et al. 2004, Hailer et al. 2012, Miller et al. 2012). Thus, the brown and polar bear lineages are relatively young.

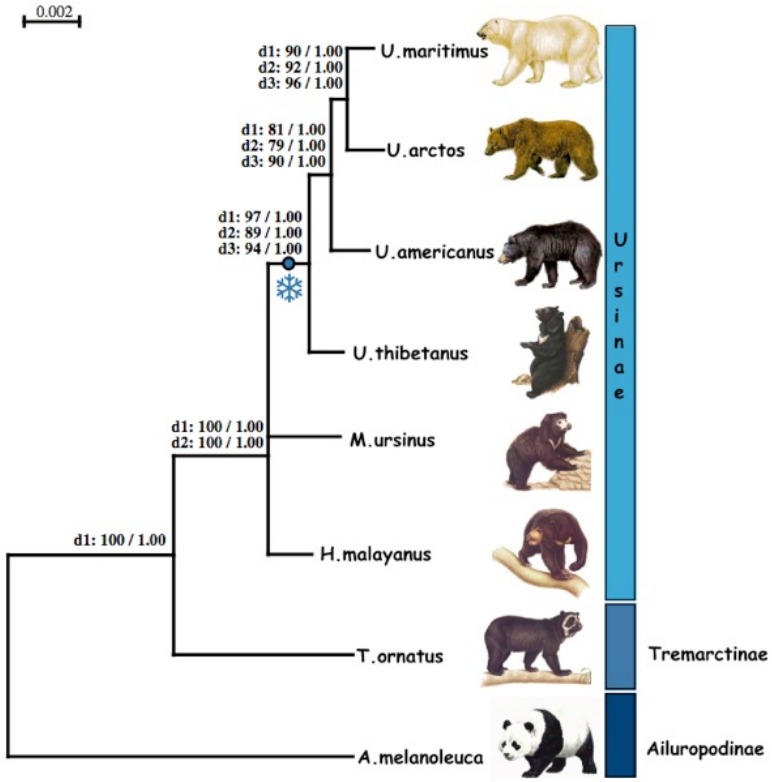

Figure 1: Ursidae family phylogeny using Bayesian and maximum likelihood analysis on 14 combined nuclear genes using giant panda as an outgroup. The numbers above the branches represent posterior probabilities obtained from datasets. The snowflake represents the species that hibernate. Figure from Pagès et al. 2008.

1.2 *Ursus maritimus* distribution

While some bear species have wide geographical distributions, polar bears can only be found in the northern hemisphere with a largely circumpolar range (Amstrup 2003). They are considered highly adapted artic mammals limited in range to areas that are largely covered by ice all year round (Rah et al. 2005, Hailer et al. 2012). They have successfully inhabited almost all sea ice habitats throughout the circumpolar artic and estimates of the global polar bear population ranges between 21,500-25,000 individuals in 19 genetically indistinguishable populations (Figure 2) (Paetkau et al. 1999, Derocher et al. 2004). Polar bears, unlike the other Ursids, (*Ursus arctos* and *Ursus americanus*) are not territorial probably due to the variability of prey density within their ranges and thus, the energetic costs of protecting a territory are not worth any potential benefit (Kazlowski and Stirling 2010). They are

estimated to travel around 5,000 km per year with a mean movement rate of ~ 0.72 km/h covering a range around 300,000 square kilometers (Øystein et al. 2003, Laidre et al. 2012, Kazlowski and Stirling 2010). Despite wide geographic distribution within the artic, polar bear population numbers are declining. The two major factors for this observed reduction are both legal harvest and global warming. Legal harvesting causes the death of around 1,000 animals per year (approximately 4% of the entire polar bear population). The major problem for the polar bear population, however, is global warming that is currently destroying the arctic ecosystem by diminishing the ice coverage and increasing the sea level. The lack of ice is pushing the polar bear population away from their natural environment and food sources, which consists mainly of seals. Since 2008, polar bears have been place on the IUCN Red List of threatened species under the category of vulnerable reflecting an effort to avoid their predicted extinction in the next 100 years (Edwards et al. 2011, Derocher et al. 2004, Briggs 2001).

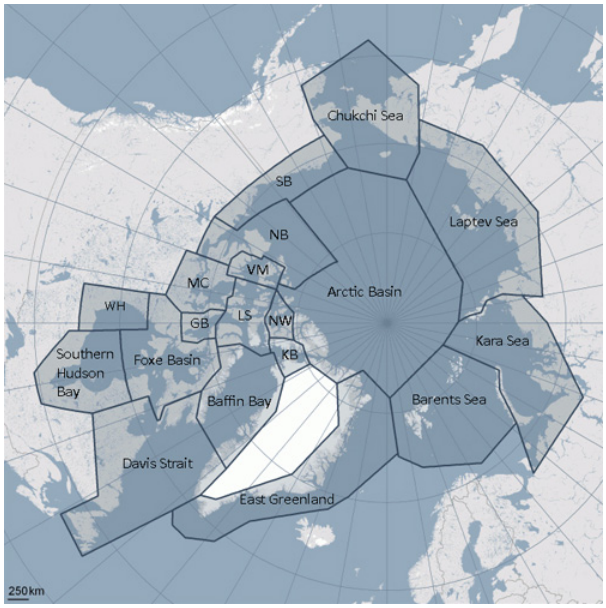

Figure 2: Arctic map that indicates the locations of the 19 population of polar bears. Based on IUCN data there is no known information about the population size in Chukchi Sea, Laptev Sea, Kara Sea, East Greenland and the Artic Basin. The Barent Sea has an estimated population ~ 2650, Davis Strait ~ 2150, Baffin bay ~2074, Kane basin (KB) ~150, Norwegian Bay (NW) ~190, Lancaster Sound (LS) ~ 2500, Gulf of Boothia (GB) ~1600, Foxe Basin ~2300, ~ Southern Hudson Bay ~900, Western Hudson Bay (WH) ~935,

M'Clintock Channel (MC) ~284, Viscount Melville Sound (VM) ~160, Northern Beaufort Sea (NB) ~1200, Southern Beaufort Sea (SB) ~1526. The map was taken from IUSN/SSC PBSG.

1.3 Polar bear morphology

Constantine Philips, a British naval officer, was the first to scientifically describe polar bears in 1774 (Ovsyanikov 1998). *Ursus maritimus* is the largest of all eight members of the Ursidae family, and the largest non- aquatic carnivore in the world. Adult males weigh up to 650 kg and measure between 2.1-2.7 m, while females range between 150-250 kg and can reach up to 2.1 m in height (Kazlowski and Stirling 2010 Rah et al. 2005, Stirling, I 1998) (Figure 3). Polar bear body architecture follows the Allen's rule, which states that extremity length decreases with increasing latitude (Alho et al. 2010), with their smaller extremities (ears and tails) adapted to cold climates (Derocher 2012). They are very good swimmers, most likely due to their large paddle like paws. An uninterrupted swim of 65 km has been recorded, as has a dive of 2-minute duration (Ovsyanikov 1998, Briggs 2001, Kazlowski and Stirling 2010).

Figure 3: Captive male polar bear, Lars, in Wuppertal Zoological Garden. Picture is courtesy of Barbara Scheer.

Polar bears are completely covered with fur with nose being the only exception (Amstrup 2003). The pelage of a polar bear has a distinct white-yellow color due to the lack of pigments. Their fur is divided into two layers; the underfur which is fine, short (5 cm), dense, and its purpose is to conserve heat while the bears swim in cold water. The second layer, which is composed of guard hairs, is long (16 cm) and coarser with a hollow center that acts as insulation. Beneath the yellowish white fur the species has black skin in contrast with other bear species that have pink skin. Black skin is an adaptation of the polar bear with an unknown function (Derocher 2012). Underneath the skin there is a 10 cm think layer of vascularized adipose tissue an adaptation that provides further thermal insulation. The described adaptations help the animals withstand winter temperatures below -30 C that are common in the artic (Garner et al. 1990, Derocher 2012).

1.4 Hunting and Diet

Polar bears are the only members of the Ursidae that are exclusively carnivorous with specialized craniodental adaptations to accommodate their diet habits (Slater et al. 2010). Due to their marine mammal diet and the fact that they are most often found on sea ice, polar bears are considered to be ecologically marine mammals (Vongraven et al. 2012). Their main prey consists of two species of phocid-seals: ringed seals (*Phoca hispida*), and bearded seals (*Erignathus barbatus*) but they also eat harp seals (*Phoca groenlandica*), walruses (*Odobenus rosmarus*) and whales such as white whales (*Delphinapterus leucas*) and narwals (*Monodon monoceros*) (Paetkau et al. 1999, Derocher et al. 2004, Laidre et al. 2012). In general, polar bears are considered opportunistic feeders and will eat any meat source available in their environment including carcasses of larger whales, and other polar bears in order to fulfil their energy requirements (Derocher 2012). Several hunting adaptations aid polar bears in the capture of marine prey. They have shorter and more curved claws than other bears, which is an adaptation that aids the movement on ice and is more effective in capturing slippery prey like seals. Their body is more streamlined and elongated, a modification that allows them to lunge deep into ice holes and capture seals that attempt to retreat (Ovsyanikov 1998, Kazlowski and Stirling 2010). Their white-yellowish fur also provides an excellent camouflage (Derocher 2012).

1.5 Mating and Sexual Dimorphism

Polar bears, like all bears, are *K*-selected species with small litter size (1.7 cubs), high maternal investment, and high adult survival rate (Bunnel and Tait 1981, Vongraven et al. 2012). However, unlike other bear species, they do not have a fixed habitat range, so finding a suitable partner for reproduction can be challenging (Derocher 2012). Male polar bears are sexually mature at age 3 but unlikely to reproduce until age 6 due to the competition with larger older males (Rosing-Asvid et al. 2002). Females on the other hand, reach sexual maturity at the age of 4 and start reproducing from that age until the end of their lives with an interbirth interval of 2-3 years due to long nurturing periods of their cubs (Sonne et al. 2007, Derocher 2012). This prolonged period of nurturing creates a skewed sex ratio in available partners with fewer females than males reproductively active at any given time (Ramsay and Stirling 1986). The skewed ratio increases intraspecies sexual competition with the bigger males having an advantage which promotes sexual dimorphism of the species (Ramsay and Stirling 1986, Derocher 2012). All bear species show some evidence of sexual dimorphism. In polar bears it is extreme with male polar bears being twice as big, on average, as females. Sexual dimorphism in polar bears is also observable in the jaw and skull with male polar bears having both longer molars and longer, higher skull sagittal crests (Derocher 2012).

1.6 Denning

Black and brown bears den for prolonged periods during winter when food sources are scarce in an effort to conserve energy. Polar bears do not hibernate for extended periods even though they are close relatives of brown bears. Lack of hibernation indicates that even during the winter there is no food scarcity in the artic (Derocher 2012). Even when food sources are limited, polar bears do not go into an extended hibernation. Instead they reduce their metabolic rate, reduce their heart rate, start using their fat storage, recycle proteins, and enter into a state called walking hibernation (Ellis 2009, Derocher 2012). The only reason for a polar bear to go into a denning phase over winter is when the animal is pregnant.

Female polar bears go into denning before giving birth and can stay in hibernation for as much as 8 months without drinking, eating, urinating or defecating (Ellis 2009, Derocher

2012). This hibernation period is necessary for the successful birth and development of the cub. Polar bear newborn cubs are extremely small weighing approximately 0.5-0.7kg (Ellis 2009, Derocher 2012), so the mother must create the necessary conditions to regulate the environment and temperature to ensure the newborns survival (Durner et al. 2002, Fischbach et al. 2007, Derocher 2012). A warm den environment is associated with reduced energy consumption, which is very important for the mother since during denning she does not have access to food. Once the cub weighs around 15 to 20 pounds and is ready to travel, or when the fat storage of the mother is diminished the polar bear exits the den (Ellis 2009).

A few months after the den exit, the mother continues nursing the cubs but after approximately 4 months the cubs are weaned. Once the cub reaches around 2.5 years of age and is large enough to hunt by itself, the cub and mother separate, freeing the mother to reproduce again (Ellis 2009).

1.7 *Ursus maritimus* in captivity

There are approximately 336 polar bears in captivity in zoos worldwide (Linke 2011). The majority represents wild introductions that were brought to zoos with no conservation program in mind. As global warming, hunting, and other factors have reduced the polar bear population these captive animals provide an excellent conservation resource (Van Dyke 2008).

Captive polar bears are enclosed in areas that must obey the regulations of the polar bear protection act. The enclosures should include the main exhibit area, off exhibit area, cubbing den, pool, and access to fresh water at all times. The main exhibit enclosures should have a minimum area of 500 m^2 plus 150 m^2 per additional animals, while the off exhibit enclosure should be 75 m^2 plus 25 m^2 per additional animal. The habitat in the exhibits should resemble as close as possible the actual living environment of the animals in the wild. The pool should have minimum area of 70 m^2 and a minimum depth of 3 m. The cubbing den is essential for pregnant females; it needs to be in a quiet area away from the main exhibit and should have minimum dimensions of 2.5 m x 2.5 m x 2.5 m (AZA Bear Tag 2009). All the areas of the enclosure except the cubbing den should provide the animal with the capability of running, walking, daily variability in its routine and general stimuli to help minimize

stereotypy behaviors that are observed in many captive animals, polar bears in particular (Briggs 2001, AZA Bear Tag 2009, Derocher 2012).

Wild polar bear diet is primarily seal blubber and skin that has a high concentration of fat. Captive polar bears main food source, in contrast, is dog food, omnivore food pallets, carcasses, heads and feet of pigs and some enrichment food like raisins and peanut butter (Briggs 2001, AZA Bear Tag 2009).

Physiological comparison of wild life and captive polar bear populations revealed that wild polar bears have lower hemoglobin and hematocrit than captive polar bears, (Bossart et al. 2001), while Nelson et al. (1983) demonstrated that captive bear populations have constantly lower levels of urea and higher levels of cholesterol than their wild counterparts. These differences are likely due to the different diet and environmental conditions in captivity (Nelson et al. 1983, Briggs et al. 2001). Differences are also observed in reproductive behavior. Wild animals are usually breed from March to June, while captive counterparts exhibit courtship behavior (playing with females, and following them around), and tried to breed earlier than wild polar bears (AZA Bear Tag 2009). Despite such differences, much of their behavior mirrors that of wild polar bears. For example, they are generally solitary animals and social avoidance is observed in captivity even in cases where animals are co-housed (Renner and Kelly 2006).

The average life-span of polar bears in captivity is approximately 18 years with some animals surviving 41 years. In contrast, wild polar bear average life expectancy is 15-18 years with a maximum-recorded age of 31 years of age (Briggs 2001, AZA Bear Tag 2009).

1.8 Viruses, bacteria, and parasites detected in *Ursus maritimus* free-living populations

Polar bears are apex predator of the artic ecosystem with humans and other polar bears as their only competition. As the top predator in their region and an opportunistic feeder, polar bears are exposed to several viruses, parasites, and pathogens that can be found in their main food sources. Polar bears provide an excellent sentinel species for monitoring pathogens agents that are circulating in the artic, and particularly in marine mammals (Rah et al. 2005). Table 1 summarizes the pathogens that have been screened for thus far in polar bears across their range.

Agent	Area	Test	Reference
Trichinella	Canada, Russia, Alaska	Serological test	Rah et al. 2005
	Svalbard	Serological test	Asbakk et al. 2010
Toxoplasma gondii	Canada, Russia, Alaska	Serological test	Rah et al. 2005
	Svalbard	Serological test	Oksanen et al. 2009
Brucella sp.	Canada, Russia, Alaska	Serological test	Rah et al. 2005, O'Hara et al. 2010
	Svalbard	Serological test	Tryland et al. 2001
Canine distemper Virus	Canada	Serological test	Cattet et al. 2004
	Alaska & Russia	Serological test	Deem et al. 2000
	Svalbard	Serological test	Tryland et al. 2005
Dolphin morbillivirus	Alaska	Serological test	Tryland et al. 2005
	Svalbard	Serological test	Tryland et al. 2005
Phocine distemper Virus	Canada	Serological test	Cattet et al. 2004
	Svalbard	Serological test	Tryland et al. 2005
Porpoise morbillivirus	Alaska & Russia	Serological test	Garner et al. 2000
Rabies	Canada	Immunohistochemistry & mouse inoculation test	Taylor et al. 1991
Calicivirus	Svalbard	Serological test	Tryland et al. 2005

Table 1: Parasite, viruses, and bacterial screenings performed on wild polar bear samples

Polar bears are apparently not easily infected by parasites (Schliebe et al. 2006). Nonetheless Trichinella, a nematode that is able to infect many mammals was present in approximately 60% of all wild polar bear populations (Asbak et al. 2010, Follmann et al. 1996, Rah et al. 2005, Tryland et al. 2001). Despite the high number of seropositive animals, infection is not associated with morbidity or mortality (Schliebe et al. 2006). *Toxoplasma gondii* is also another mammal parasite that was detected in polar bears with prevalence around 6% but like *Trichinella* has no apparent effect on animal health (Schliebe et al. 2006, Rah et al. 2005).

Brucella sp. is considered an important disease causing gram-negative bacteria that infects cattle, swine and humans. Infection can lead to reproductive disorders. Several marine mammals, including ringed seals, one of the major food sources of polar bears, have tested positive for this bacterium (Tryland et al. 2001). Polar bears were screened demonstrating antibodies against Brucella in approximately 5% of the polar bear population (Tryland et al. 2001, Rah et al. 2005, O'Hara et al. 2010). Even though this bacterium was shown to cause abortion in other marine mammals, no such effects have been recorded for polar bears.

Four morbilliviruses are currently known to be in circulation in the artic: Canine distemper virus (CDV), phocine distemper virus (PDV), dolphin morbillivirus (DMV), and porpoise morbillivirus (PMV) (Garner et al. 2000). Morbilliviruses are single stranded RNA viruses that periodically emerge in the artic causing mass mortalities in seals. These viruses also infect other artic carnivores such as the artic fox (Follman et al. 1996, Tryland et al. 2005). Polar bear interactions with the infected species likely provide a link to the circulation and ecology of morbilliviruses in the arctic (Garner et al. 2000). Serological screening of several populations of polar bears revealed that 8-45% of the animals had antibodies that reacted with the morbilliviruses, with CDV having the highest immunogenic response (Follman et al. 1996, Garner et al. 2000, Cattet et al. 2004, Tryland et al. 2005). The high antibody prevalence in polar bears could indicate host-virus co-adaptation resulting in limited or no health consequences for the host. Furthermore, polar bears could serve as potential reservoir and source of infection for morbilliviruses in the artic ecosystem (Garner et al. 2000).

Caliciviruses infect seals and walruses. A recent study revealed that 2% of tested polar bears were exposed to a calicivirus with no clear indication about the strain (Tryland et al. 2005). Almost all microorganisms identified in polar bears have no apparent effect on the species. The Rabies virus is an ssRNA virus of the family Rhabdoviridae that has the artic fox as its main vector in the arctic (Tordo et al. 1986, Prestrud et al. 1992). There is only one confirmed case of rabies infection in polar bears in which the animal suffered from posterior paralysis. Necropsy of the animal did not reveal an injury that could have caused the observed paralysis. Infection with rabies virus is what most likely caused the animals loss of posterior

motor control (Taylor et al. 1991). Thus, in contrast to most other detected microorganisms, rabies can cause disease in polar bears.

1.9 Viruses and parasites detected in captive polar bears

Captive polar bears unlike their wild counterparts are under the rules of the polar bear care manual that is employed by all zoos (AZA Bear Tag 2009). All transferred captive and wild caught bears are placed in quarantine for 30 days before entering the zoo enclosure. This allows the bears to adjust to a new environment and to minimize the risk of potential pathogen transmission to other animals. The 30-day quarantine period can be extended in case the animal demonstrates signs of infection. During the isolation period the animal goes through a de-worming process, complete physical examination, ecto and endoparasite evaluation, serum collection for extended serology testing, urine analysis, and recommended vaccination for rabies, leptospirosis, and tetanus toxoid. Once the individual has tested negative for all the known pathogens that infect polar bears in the wild (Table 1) and the individual has three negative fecal exams, it is released into the polar bear enclosure (AZA Bear Tag 2009). Despite all the protective measures that zoos use to avoid and protect polar bears from infections, there are still pathogens detected in captive polar bears in many parts of the world (Table 2).

Agent	Area	Test	Reference
Trichinella	Mexico City Zoo	Western blot – Serological test	Yepez-Mulia et al 1996
	Knoxville Zoo	Necropsy	Sleeman et al. 1994
Bordetella bronchiseptica	Lincoln park zoo	Bacterial culture	Lacasse et al. 2006
Suid Herpesvirus 1	Circus -Spain	Polymerase Chain Reaction	Banks et al. 1999
Equine Herpesvirus 9	San Diego Zoo	Polymerase Chain Reaction	Schrenzel et al. 2008
West Nile Virus	Toronto Zoo	RT-PCR Serology	Dutton et al. 2009

Table 2: Parasite, viral, and bacterial agents observed in captive polar bears

Trichinella infections, which have approximately 60% prevalence in wild populations, also appear to occur in captivity with no apparent health effect. The parasite was detected in two captive polar bears, one in Mexico City, Mexico and one in Knoxville, USA during necropsy. Both of the bears were wild caught and spent the majority of their lives in captivity. *Post mortem* necropsy revealed the presence of Trichinella larvae infection that could have been acquired in the wild. However, rodents in the zoos or the meat fed to the animals could also have been a source of infection (Sleeman et al. 1994, Yepez-Mulia et al 1996).

Herpesvirales is a group of large double stranded DNA viruses with distinct virion architecture (McGeoch et al. 2006, Davison et al. 2009). Herpesviruses are further divided into three suborders: *Alloherpesviridae*, which contains the fish and frog herpes viruses, *Malacoherpesviridae*, which has the newly discovered bivalve herpes virus as its only member, and *Herpesviridae*, which includes the mammalian, bird, and reptile viruses (Davison et al. 2009). The Herpesviridae family is further subdivided into three groups based on biological criteria (host range and spectrum of cells that support viral latency): the alpha, beta, and gammaherpesviruses. The three subgroups contain 90% of all discovered mammalian herpesviruses to date (Davison et al 2009, Ackermann et al. 2004). Different alphaherpesviruses have been shown to infect polar bears in captivity (Banks et al. 1999, Schrenzel et al. 2008).

The first case involved four circus polar bears in Spain that were infected with the suid herpesvirus 1, a virus that primarily infects pigs and causes what is called Aujeszkys disease (Banks et al. 1999). Aujeszkys disease is an economically important disease of pigs that can cause morbidity and mortality in young swine. When suid herpesvirus 1 or pseudorabies infects a non-swine related species it usually results in mortality regardless of age (Marchioli et al. 1987). Four polar bears were found to be infected with suid herpesvirus 1 and three died over a 16 day period. It was postulated that the source of infection was pig meat given to the bears (Banks et al. 1999).

Another case of alphaherpesvirus infections was documented in the San Diego Zoo involving a 12-year-old polar bear. The bear exhibited progressive neurological signs and did not respond to therapy and eventually was euthanized. Necropsy of the bear showed

nonsuppurative meningoencephalitis with neuronal and glial intranuclear inclusion bodies. Polymerase chain reaction screening of polar bear tissues revealed that the likely causative agent was Equine Herpes Virus 9 (EHV-9) (Schrenzel et al. 2008) (Figure 4). This herpes virus is the newest member of the Equine herpesvirus family with potential for wide host cross species transmission for example it was previously reported as the likely causal agent for encephalitis in a giraffe (Schrenzel et al. 2008, Fukushi et al. 2012).

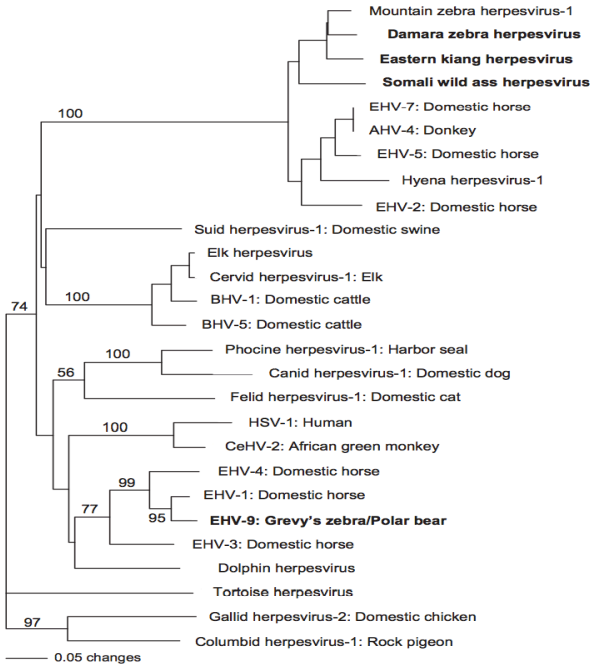

Figure 4: Phylogram of herpesviruses polymerase predicted amino acid segment with polar bear sequence being one of the highlighted sequences. Figure taken from Schrenzel et al. 2008

Single stranded RNA viruses of the *Flavivirus* genus have also infected and caused clinical symptoms in captive polar bears. In the Toronto Zoo, a 26-year-old polar bear presented with partial paralysis and deteriorating condition leading the zoo veterinarians to euthanize the animal two days after the onset of the symptoms. Serological and molecular tests indicated that the animal was infected with West Nile Virus; a single stranded RNA virus

within the family *Flaviviridae* that is transmitted by mosquitoes (Kramer et al. 2007, Dutton et al. 2009).

Bacterial infection was also detected in captive polar bears but without causing fatalities. A 5-year-old male bear in the Lincoln Park Zoo presented partial anorexia and depression. Sedation was performed to obtain blood samples, fecal samples, and for general physical examination. Blood work and physical examination did not reveal signs of infection but bacteria culture yielded *Bordetella bronchiseptica*, which is a gram-negative bacterium that is considered to be a respiratory pathogen in many species. This bacterium is transmitted through aerosol or by direct contact and it has many environmental reservoirs. The polar bear recovered after treatment but zoo officials were unable to detect the source of infection (Lacasse et al. 2006).

2. Study Aims

Between June 2010 and March 2011 three polar bears, Knut, Lars and Jerka, located in two different zoos suffered from epileptic seizures resulting in the deaths of Knut and Jerka, while the third polar bear Lars survived after medical intervention. The 22-year-old female Jerka from the Zoological Garden Wuppertal in Germany first presented epileptic symptoms and died despite medical intervention. Necropsy of Jerka indicated that the cause of death was encephalitis of unknown etiology. Knut from the Berlin Zoological Garden exhibited seizures that resulted in his falling into the enclosure moat and drowning. Knut's necropsy revealed that the most likely cause of the observed seizures was non-suppurative panmeningoencephalities of unknown etiology. The aims of this study were to develop and apply methods to characterize the causative pathogens in Knut and Jerka. In this thesis I describe in chapter II the pipeline and methods that were used to identify the likely causative agents of the two polar bear deaths. Protocols were developed that could have a wide applicability in a variety of wildlife and captive animals where little is known about the pathogens that infect them. Chapter III and chapter IV include detailed analysis of two findings obtained through the use of the pipeline described in chapter II. First is the description of a recombinant equine herpes virus 1 that was identified as the most likely causative agent in the death of Jerka the polar bear from the Wuppertal Zoological Garden. The results refute the

dogma of herpesvirus species specificity and raise important questions about viral transmission. Second is the discovery and characterization of a novel endogenous betaretrovirus group that was identified in Knut and Jerka and is present in all bears. Evolutionary analysis of this novel viral group demonstrates a complex evolutionary history of betaretroviruses in mammals

3. References

Ackermann, M. (2004). Herpesviruses. In "*Bacterial Artificial Chromosomes"* (S. Zhao & M. Stodolsky, Eds), pp.199-219. Humana Press, New Jersey.

Alho, J. S., Herczeg, G., Laugen, A. T., Rasanen, K., Laurila, A., & Merila, J. (2010). Allen's rule revisited: quantitative genetics of extremity length in the common frog along a latitudinal gradient. *Journal of Evolutionary Biology.* **24**: 59-70.

Amstrup, S. (2003). Polar Bear. In: Wild Mammal of North America. Biology, Management, and Economics, pp. 587-610. John Hopkins Press, Baltimore, MD.

Åsbakk, K., Aars, J., Derocher, A. E., Wiig, Ø., Oksanen, A., Born, E. W., Dietz, R., Sonne, C., Godfroid, J., & Kapel, C. M. (2010). Serosurvey for *Trichinella* in polar bears (*Ursus maritimus*) from Svalbard and the Barents Sea. *Veterinary parasitology*, **172**: 256-263.

AZA Bear TAG (2009). Polar Bear (*Ursus maritimus*) Care Manual. Association of Zoos and Aquariums, Silver Spring, MD.

Banks, M., Monsalve-Torraca, L. S., Greenwood, A.G., & Taylor, D. C. (1999). Aujeszky's disease in captive bears. *Veterinary Record*. **145**: 362-365.

Briggs, M. B. (2001). Polar bears. In " CRC Handbook of marine mammal medicine: health, disease, and rehabilitation" (L. Dierauf & M. D. Gulland, Eds), 2nd edition, pp.989-1004. CRC press, New York.

Bossart, G. D., Reidarson, T. H., Dierauf, L. A., & Duffield, D. A. (2001). Clinical pathology. In " CRC Handbook of marine mammal medicine: health, disease, and rehabilitation" (L. Dierauf & M. D. Gulland, Eds), 2nd edition, pp.383-436. CRC press, New York.

Bunnell, F. L., & Tait, D. E. N. (1981). Population dynamics of bears – implications. In " Dynamics of large mammal populations" (C. W. Fowler & T. D. Smith, Eds.), pp.75-98. John Wiley and Sons, New York.

Cattet, M. R., Duignan, P. J., House, C. A., & Aubin, D. J. S. (2004). Antibodies to canine distemper and phocine distemper viruses in polar bears from the Canadian Arctic. *Journal of wildlife diseases*. **40**: 338-342.

Davison, A. J., Eberle, R., Ehlers, B., Hayward, G. S., McGeoch, D. J., Minson, A. C., Pellet, P. E., Roizman, B., Studdert, M. J., & Thiry, E. (2009). The order herpesvirales. *Archives of virology*, **154**: 171-177.

Deem, S. L., Spelman, L. H., Yates, R. A., & Montali, R. J. (2000). Canine distemper in terrestrial carnivores: a review. *Journal of Zoo and Wildlife Medicine*. **31**: 441-451.

Derocher, A. E., Lunn, N. J., & Stirling, I. (2004). Polar bears in a warming climate. *Integrative and Comparative Biology*. **44**: 163-176.

Derocher, A. E. (2012). Polar Bears: A complete guide to their biology and behavior. John Hopkins University Press, Baltimore.

Durner, G. M., Amstrup, S. C., & Fischbach, A. S. (2003). Habitat characteristics of polar bear terrestrial maternal den sites in northern Alaska. *Arctic*. **54**: 55-62.

Dutton, C. J., Quinnell, M., Lindsay, R., DeLay, J., & Barker, I. K. (2009). Paraparesis in a polar bear (*Ursus maritimus*) associated with West Nile virus infection. *Journal of Zoo and Wildlife Medicine*. **40**: 568-571.

Edwards, J. C., Suchard, M. A., Lemey, P., Welch, J. J., Barnes, I., Fulton, T. L., Barnett, R., O'Connell, T. C., Coxon, P., Monoghan, N., Valdiosera, C. E., Lorenzen, E. D., Willerslev, E., Baryshnikov, G. F., Rambaut, A., Thomas, M. G., Bradley, D. G., & Shapiro, B. (2011). Ancient hybridization and an Irish origin for the modern polar bear matriline. *Current Biology*. **21**: 1251-1258.

Ellis, R. (2009). On thin ice: The changing world of the polar bear. Alfred A. Knopf, New York.

Fischbach, A. S., Amstrup, S. C., & Douglas, D. C. (2007). Landward and eastward shift of Alaskan polar bear denning associated with recent sea ice changes. *Polar Biology*. **30**: 1395-1405.

Fukushi, H., Yamaguchi, T., & Yamada, S. (2012). Complete genome sequence of equine herpesvirus type 9. *Journal of Virology*. **86**: 13822.

Follmann, E. H., Garner, G. W., Evermann, J. F. & McKeirnan, A. J. (1996) Serological evidence of morbillivirus infection in polar bears (*Ursus maritimus*) from Alaska and Russia. *Veterinary Record*, **138**: 615-618.

Garner, G. W., Knick, S. T., Douglas, D. C. (1990). Seasonal movements of adult female polar bears in the Bering and Chukcki Seas. *International Conference Bear Research and Management,* **8**: 219-226.

Garner, G. W., Evermann, J. F., Saliki, J. T., Follmann, E. H., & McKeirnan, A. J. (2000). Morbillivirus ecology in polar bears (*Ursus maritimus*). *Polar Biology*, **23**: 474-478.

Hailer, F., Kutschera, V. E., Hallstrom, B. M., Klassert, D., Fain, S. R., Leonard, J. A., Arnason, U., & Janke, A. (2012). Nuclear genomic sequences reveal that polar bears are an old and distinct bear lineage. *Science* **336**: 344-347.

Kramer, L. D., Li, J., & Shi, P. Y. (2007). West Nile virus. *Lancet Neurology*. **6**: 171-181.

Kazlowski, S., & Stirling, I. (2010). Ice Bear: The Artic World of Polar bears. The Mountaineers Books.

Lacasse, C., & Gambie, K. C. (2006). Tracheitis associated with *Bordetella bronchiseptica* in a polar bear (*Ursus maritimus*). *Journal of Wildlife Medicine*. **37**: 190-192.

Laidre, K. L., Born, E. W., Gurarie, E., Wiig, Ø., & Stern, H. (2012). Females roam while males patrol: divergence in breeding season movements of pack-ice polar bears (*Ursus maritimus*). *Proceedings of the Royal Society B*. **280**: 20122371.

Marchioli, C. C., Yancey, R. J., Petrovskis, E. A., Timmins, J. G., & Post, L. E. (1987). Evaluation of pseudorabies virus glycoprotein gp50 as a vaccine for Aujeszky's disease in mice and swine: expression by vaccinia virus and Chinese hamster ovary cells. *Journal of Virology*. **61**: 3977-3982.

McGeoch, D. J., Rixon, F. J., & Davison, A. J. (2006). Topics in herpesvirus genomics and evolution. *Virus research*, **117**: 90-104.

Miller, W., Shuster, S. C., Welch, A. J., Ratan, A., Bedoya-Reina, O. C., Zhao, F., Kim, H. L., Burhans, R. C., Drautz, D. I., Wittekindt, N. E., Tomsho, L. P., Ibarra-Laclette, E., Herrera-Estrella, L., Peacock, E., Farley, S., Sage, G. K., Rode, K., Obbard, M., Montiel, R., Bachmann, L., Ingolfsson, O., Aars, J., Mailund, T., Wilg, O., Talbot, S. L., & Lindqvist, C. (2012). Polar and brown bear genomes reveal ancient admixture and demographic footprints of past climate change. *PNAS* **109:** 14295-14296.

Nelson, R. A., Folk Jr, G. E., Pfeiffer, E. W., Craighead, J. J., Jonkel, C. J., & Steiger, D. L. (1983). Behavior, biochemistry, and hibernation in black, grizzly, and polar bears. *Bears: Their Biology and Management*. **5**: 284-290.

O'Hara, T. M., Holcomb, D., Elzer, P., Estepp, J., Perry, Q., Hagius, S., Kirk, C. (2010). Brucella species survey in polar bears (*Ursus maritimus*) of Northern Alaska. *Journey of Wildlife Diseases*. **46**: 687-694.

Oksanen, A., Åsbakk, K., Prestrud, K. W., Aars, J., Derocher, A. E., Tryland, M., Wiig, Ø., Dubey, J. P., Sonne, C., Dietz, R., Andersen, M., & Born, E. W. (2009). Prevalence of antibodies against *Toxoplasma gondii* in polar bears (*Ursus maritimus*) from Svalbard and East Greenland. *Journal of Parasitology*. **95**: 89-94.

Ovsyanikov, N. (1998). Polar bears. (J. Billinghurst Ed.). Voyager Press, Stillwater, MN.

Øystein, W., Born, E. W., Pedersen, & Pedersen, L. T. (2003). Movements of female polar bears (*Ursus maritimus*) in the East Greenland pack ice. *Polar Biology*. **26**: 509-516

Paetkau, D., Amstrup, S. C., Born, E. W., Calvert, W., Derocher, A. E., Garner, G. W., Messier, F., Stirling, I., Taylor, M. K., Wiig, Ø., & Strobeck, C. (1999). Genetic structure of the world's polar bear populations. *Molecular Ecology*. **8**: 1571-1584.

Pagès, M., Calvignac, S., Klein, C., Paris, M., Hughes, S., & Hänni, C. (2008). Combine analysis of fourteen nuclear genes refines the *Ursidae* phylogeny. *Molecular Phylogenetics and Evolution* **47**: 73-83.

Prestrud, P., Krogsrud, J., & Gjertz, I. (1992). The occurrence of rabies in the Svalbard Islands of Norway. *Journal of wildlife diseases*, **28**: 57-63.

Rah, H., Chomel, B. B., Follmann, E. H., Karsten, R. W., Hew, C. H., Farver, T. B, Garner, G. W., & Amstrup, S. C. (2005). Serosurvey of selected zoonotic agents in polar bears (*Ursus maritimus*). *Veterinary Record* **156**: 7-13.

Ramsay, M. A., & Stirling, I. (1986). On the mating system of polar bears. *Canadian Journal of Zoology*. **64**: 2142-2151.

Renner, M., & Kelly, A. (2006). Behavioral decisions for managing social distance and aggression in captive polar bears. *Journal of Applied Animal Welfare Science*. **9**: 233-239.

Rosing-Asvid, A., Born, E. W., & Kingsley, M. C. S. (2002). Age at sexual maturity of males and timing of the mating season of polar bears (*Ursus maritimus*) in Greenland. *Polar Biology*. **25**: 878-883.

Schliebe, S., Evans, T., Johnson, K., Roy., M., Miller, S., Hamilton, C., Meehan, R., Jahrsdoerfer, S. (2006). Range wide status review of the polar bear (*Ursus Maritimus*). U.S. Fish and Wildlife Service, Anchorage, AK.

Shrenzel, M. D., Tucker, T. A., Donovan, T. A., Busch, M. D. M., Wise, A. G., Maes, R. K., & Kiupel, M. (2008). New Hosts for equine herpesvirus 9. *Emerging Infectious Diseases*. **14**: 1616-1619.

Slater, G. J., Figueirido, B., Louis, L., Yang, P., & Van Valkenburgh, B. (2010). Biomechanical consequences of rapid evolution in the polar bear lineage. *PLoS ONE*. **5**: e 13870.

Sleeman, J. M., Ramsay, E. C., Faulkner, C. T., Patton, S. and Mason, G., 1994. Trichinosis in a polar bear *(Ursus maritimus).* Proceedings of the American Association of Zoo Vets, pp. 352-353.

Sonne, C., Dietz, R., Born, E. W., Riget, F. F., Leifsson, P. S., Bechshøft, T. Ø., & Kirkegaard, M (2007). Spatial and temporal variation in size of polar bear (*Ursus maritimus*) sexual organs and its use in pollution and climate change studies. *Science of the Total Environment*. **387**: 237-246

Stirling, I. (1998). Polar bears. University of Michigan Press. Ann Arbor, MI

Taylor, M., Elkin, B., Maier, N., & Bradley, M. (1991). Observation of a polar bear with rabies. *Journal of Wildlife Diseases*. **27**: 337-339.

Tordo, N., Poch, O., Ermine, A., Keith, G., & Rougeon, F. (1986). Walking along the rabies genome: is the large GL intergenic region a remnant gene?.*Proceedings of the National Academy of Sciences*, **83**: 3914-3918.

Tryland, M., Derocher, A. E., Wiig, Y., & Godfroid, J. (2001). Brucella sp. antibodies in polar bears from Svalbard and the Barents Sea. *Journal of Wildlife Diseases*. **37**: 523-531.

Tryland, M., Neuvonen, E., Huovilainen, A., Tapiovaara, H., Osterhaus, A., Wiig, Ø., & Derocher, A. E. (2005). Serologic survey for selected virus infections in polar bears at Svalbard. *Journal of Wildlife Diseases*. **41**: 310-316.

Van Dyke, F. (2008). Conservation Biology: Foundation, Concepts, Applications. 2nd Edition. McGraw Hill, New York.

Vongraven, D., Aars, J., Amstrup, S., Atkinson, S. N., Belikov, S., Born, E. W., DeBruyn, T. D., Derocher, A. E., Durner, G., Gill, M., Lunn, N., Obbard, M. E., Omelak, J., Ovsyanikov, N., Peacock, E., Richardson, E., Sahanatien, V., Stirling, I., & Øystein, W. (2012). A circumpolar monitoring framework for polar bears. *Ursus* (*International Association for Bear Research and Management*). ISSN 1537-6176. **5**: 1-66.

Yépez-Mulia, L., Arriaga, C., Pena, M. A., Gual, F., & Ortega-Pierres, G. (1996). Serologic survey of trichinellosis in wild mammals kept in a Mexico City Zoo. *Veterinary parasitology*, **67**: 237-246.

Yu, L., Li, Q. W., Ryder, O. A., & Zhang, Y. P. (2004). Phylogeny of the bears (*Ursidae*) based on nuclear and mitochondrial genes. *Molecular Phylogenetics and Evolution* **32**: 480-494.

Yu, L., Li, Y. W., Ryder, O. A., & Zhang, Y. P. (2007). Analysis of complete mitochondrial genome sequences increases phylogenetic resolution of bears (*Ursidae*), a mammalian family that experienced rapid speciation. *BMC Evolutionary Biology* **7**: 198.

Chapter II

Polar bear encephalitis: Establishment of a comprehensive next-generation pathogen analysis pipeline for captive and free-living wildlife

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Supplementary Material

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Chapter III

A Potential Fatal Mix of Herpes in Zoos

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Chapter IV

A novel endogenous betaretrovirus group characterized from polar bears (*Ursus Maritimus*) and giant panda (*Ailuropoda melanoleuca*)

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Chapter V
Concluding remarks

Concluding remarks

Polar bears are charismatic artic predators threatened by climate change (Rockwell and Gormezano 2009). The IUCN has listed them among vulnerable species due to the drastic decline of their population over the last decades with predicted extinction estimation in the next 100 years (Briggs 2001, Derocher et al. 2004). As a marquee species in many zoos, polar bears attract public attention and are used as ambassadors for conservation efforts of endangered wildlife. They have high visibility and can draw public attention (Renner and Kelly 2006). Despite the interest by the public, little is known about the general infection biology and pathogens that infect polar bears in the wild and in captivity, making health monitoring difficult if not impossible (Aguirre et al. 2012).

In this thesis, investigation on the cause of death of two captive polar bears led to the development of a three-stage pipeline (Chapter II), the discovery of recombinant herpesvirus that is infecting polar bears (Chapter III), and a novel endogenous betaretrovirus group (Chapter IV). The pipeline protocols include classical and modern methods, in an effort to identify the unknown pathogens that caused encephalitis to the captive polar bears. The methods used were powerful and successful in the identification of a recombinant EHV-1 virus (Chapter III) and a novel endogenous betaretrovirus (Chapter IV). Further characterization of the endogenous retrovirus (Chapter IV) helped resolve the phylogeny of the HERV-K group, demonstrating in the process the evolutionary relationship among endogenous retroviruses from different mammals and the poor concordance of host - viral evolution that is indicative of large scale cross species transmission long ago. The pipeline results indicate that there are still major limitations to most methods when applied to non-model organisms. Even with the use of all the available methods the exact cause of Knuts' observed encephalitis could not be conclusively identified. Serological assays demonstrate that Knut had antibodies against influenza A, but unfortunately no genomic confirmation was obtained. Whether exposure to influenza was in any way associated with Knut's death is highly questionable.

Zoos all over the world define their mission as educating the public, promoting animal care, maintaining global sustainability, and conserving wild species in their exhibitions

(Clayton et al. 2009). Equids are one of the most common exhibits in the majority of zoos and EHVs are common infectious agents that are observed in equids in the wild and in captivity. Herpes viruses have been observed in all equid species tested and cause a variety of diseases and symptoms that include neonatal foal disease, rhinopneumonitis, abortion, neurological disease, and equine coital exanthema (Borchers et al. 2006, Wilson 1997, Goodman et al. 2007, Schrenzel et al. 2008, Fukushi et al. 2012). Some EHVs have also demonstrated potential to jump species and cause lethal infections in several wildlife animals in zoos around the world, an unusual property for herpesviruses which are generally host specific (Donovan et al. 2009, Wohlsein et al. 2011). In Chapter III I described a zebra EHV-1 recombinant virus that caused seizures in two polar bears, most likely resulting in the death of Jerka, while it also asymptomatically infected a third polar bear in an unrelated case in a different zoo. These results indicate that housing non-sympatric species, like polar bears and zebras in close proximity, could be problematic, as potential EHV outbreaks could be lethal for non-equids, damaging the conservation efforts of zoos. It suggests that the polar bears and other mammals affected by EHVs in captivity are inadvertently being exposed to aspects of EHV biology we do not yet understand. For example, discovering the mode of transmission in zoos will be the next future challenge to EHV related captive animal management. Part of this understanding may come from examining EHV transmission in the wild, a relatively unexplored aspect of EHV biology.

It is imperative for development of new more precise methods for wildlife diagnostics that can overcome the limitations that where observed during the viral screening of Knut. Animals in zoos are often placed in close proximity with non-sympatric species could result in further exchange of pathogens. Novel methods that are in development or have been applied in other contexts such as hybridization capture (Maricic et al. 2010) will have to be evaluated for their diagnostic potential and utility to better protect captive animals. The successes under current methodological restrictions show that once problems are recognized, intervention and further research areas can be created. Thus, the mode of transmission of EHVs in zoos needs to be investigated and is a new research area. However, in the meantime, proactive zoos can regularly screen their animals for EHVs and intervene before severe problems arise.

References

Borchers, K., Thein, P., & Sterner‐Kock, A. (2006). Pathogenesis of equine herpesvirus‐associated neurological disease: a revised explanation. *Equine veterinary journal*, *38*: 283-287.

Briggs, M. B. (2001). Polar Bears. In " CRC Handbook of marine mammal medicine: health, disease, and rehabilitation" (L. Dierauf & M.D. Gulland, Eds), 2nd edition, pp.989-1004. CRC press, New York.

Clayton, S., Fraser, J., & Saunders, C. D. (2009). Zoo experiences: Conversations, connections, and concern for animals. *Zoo Biology*, *28*(5), 377-397.

Derocher, A. E., Lunn, N. J., & Stirling, I. (2004). Polar bears in a Warming Climate. *Integrative and Comparative Biology*. **44**: 163-176.

Donovan, T. A., Schrenzel, M. D., Tucker, T., Pessier, A. P., Bicknese, B., Busch, M. D. M., Wise, A. G., Maes, R., Kiupel, M., McKnight, C., & Nordhausen, R. W. (2009). Meningoencephalitis in a polar bear caused by equine herpesvirus 9 (EHV-9). *Veterinary Pathology Online*, **46**: 1138-1143.

Fukushi, H., Yamaguchi, T., & Yamada, S. (2012). Complete genome sequence of equine herpesvirus Type 9. *Journal of Virology*. **86**: 13822.

Goodman, L. B., Loregian, A., Perkins, G. A., Nugent, J., Buckles, E. L., Mercorelli, B., Kydd, J. H., Palu, G., Osterrieder, N., & Davis-Poynter, N. (2007). A point mutation in a herpesvirus polymerase determines neuropathogenicity. *PLoS pathogens*, **3**: e160.

Maricic, T., Whitten, M., Pääbo, S., (2010). Multiplex DNA sequence capture of mitochondrial genomes using PCR products. *PLoS ONE* **5**: e14004.

Ostfeld, R. S., Tabor, G. M., House, C., & Pearl, M. C. (2002). New Directions in conservation medicine: applied cases of ecological health. (A. A. Aguirre, Ed.). Oxford University Press, New York.

Renner, M. J., & Kelly, A. L. (2006). Behavioral decisions for managing social distance and aggression in captive polar bears (*Ursus maritimus*). *Journal of Applied Animal Welfare Science*, **9**: 233-239.

Rockwell, R. F., & Gormezano, L. J. (2009). The early bear gets the goose: climate change, polar bears and lesser snow geese in western Hudson Bay.*Polar Biology*, **32**: 539-547.

Shrenzel, M. D., Tucker, T. A., Donovan, T. A., Busch, M. D. M., Wise, A. G., Maes, R. K., & Kiupel, M. (2008). New hosts for equine herpesvirus 9. *Emerging Infectious Diseases*. **14**: 1616-1619.

Wilson, W. D. (1997). Equine herpesvirus 1 myeloencephalopathy. *The Veterinary clinics of North America. Equine practice*, **13**: 53-72.

Wohlsein, P., Lehmbecker, A., Spitzbarth, I., Algermissen, D., Baumgärtner, W., Böer, M., Kummrow, M., Haas, L., & Grummer, B. (2011). Fatal epizootic equine herpesvirus 1 infections in new and unnatural hosts. *Veterinary microbiology*, **149**: 456-460.

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