

Pilot Study

I-Title and abstract: The First Phylogenetic and Spatial Analysis of Parvovirus Strains Circulating in the Serengeti Maasai Mara Ecosystem

Rationale: Despite being widely distributed among domestic and wild carnivores, little is known about the impact that canine/feline parvovirus (CPV/FPLV) has on free-living carnivore species. Parvovirus has been reported to cause mortality in the young of wild carnivores and is suspected to have triggered declines in naïve wolf populations worldwide. African wild carnivores in captivity are also susceptible to parvovirus disease, however no epidemiological research has been conducted in free-living populations.

Furthermore, the epidemiological relationship that links parvovirus infection in domestic animals and wildlife is poorly understood. Mass domestic dog vaccination campaigns (MDV) targeting parvovirus are ongoing in communities living in the periphery of the Serengeti-Maasai Mara ecosystem, but the impact that these activities are having on wild populations is not studied yet. This information is required to optimize control measures to prevent disease transmission between domestic and wild carnivores in this protected area.

Hypothesis/Objectives: The hypothesis of this study is that domestic and wild carnivores play a significant and linked role in the epidemiology of parvovirus in the Serengeti-Maasai Mara ecosystem. The objectives are: 1) to estimate the prevalence of infection in our samples and the susceptibility of different species to be infected by canine and feline parvovirus. 2) to determine which strains of parvovirus are circulating in wild and domestic carnivores living in and around this ecosystem; 3) to carry out phylogenetic analyses of these strains to assess the likelihood of cross-species transmission and the possible introduction of new strains across national borders; and 4) to evaluate the influence that proximity to human communities has on the prevalence of parvovirus infection in wild carnivores. The data from this study will lead to a broader research project that will investigate the influence that mass dog vaccination has on the ecology of parvovirus in wild and domestic carnivores.

Study design: This study is divided in four activities: 1) The detection of parvovirus infection by real-time PCR (polymerase chain reaction), in archived blood and organ samples of wild and domestic carnivore species; 2) Sequencing of the VP2 protein DNA, and phylogenetic analyses that will enable circulating parvovirus strains to be characterised; 3) Spatial analysis of the results to enable the distribution of circulating strains within the ecosystem to be mapped.

Preliminary data: In 2014 a molecular study was carried out in Spain and newly adapted PCR techniques for sequencing the whole VP2 protein DNA developed. Spatial analysis data confirmed proximity to urban areas to be a risk factor for parvovirus infection of Iberian wolves. These results support the hypothesis that parvovirus spills over into wild populations from domestic carnivores. Furthermore, research conducted in Tanzania confirmed the presence of CPV-2a and CPV-2b in wild and domestic animals, but no data is available about Feline panleukopenia virus and other strains of CPV-2.

Expected results: This study will increase our understanding of the epidemiology of parvovirus in the Serengeti-Maasai Mara ecosystem by 1) determining which parvovirus strains are currently circulating, 2) assessing the susceptibility of different carnivore species to these strains, and 3) determining the influence that proximity to human settlements has on the prevalence and distribution of different parvovirus strains among wild populations.

Budget and timeline: This study has duration of one year with a total budget of 10.800 dollars.

Potential impact for animal health: Having evaluated the epidemiology of parvovirus in the Tanzanian environment we will be better able to refine intervention measures to reduce transmission from domestic to wild carnivores. Due to the difficulties of assessing the health of litters, the impact of parvovirus on African carnivore populations is unknown. However, in captive environments large carnivores have been shown to be susceptible to parvovirus-induced disease. The introduction of new, and potentially more pathogenic, strains into the ecosystem is a potential health risk for the young of wild carnivores. Our results will enable the appropriate strain of vaccine to be selected for MDV efforts targeting communities living around the Serengeti Maasai Mara ecosystem. Additionally, subsequent research projects will build upon the data generated in this project to test the efficacy of this intervention strategy. These outputs will help to minimize the impact that human settlements, and associated domestic animals, have on the health of threatened populations of African wild carnivores.

II. Resubmission Summary:

This proposal was sent in 2014 to The Morris Foundation and we are very grateful for the response we obtained. Due to the fairly positive and encouraging comments -"This project is highly relevant in terms of wildlife health, and epidemiology of viral pathogens that can pass between domestic and wild animal hosts"- we decided to resubmit the application since our project is mandatory to assess the health and control measures currently conducted on the Serengeti Maasai Mara Ecosystem. We have introduced some changes and new explanations in the proposal, and a specific response to the comments can be found below.

Proposal Review

Brief summary of proposed research (1-2 sentences):

This molecular epidemiology study will determine the host range of parvoviruses in domestic animals (dogs specially) relative to wild carnivores in Tanzania. Parvovirus spillover-spillback has huge implications for the evolution of this virus, as other work has shown the ability of parvoviruses to evolve in novel hosts and change their host range. This has implications for vaccination strategies of domestic dogs, and the effects on wildlife.

Critique of scientific merit and proposed approach:

Stool and blood samples of domestic dogs and wildlife are available between 2002 and 2009 from the districts surrounding Serengeti National Park. First, samples will be tested for parvovirus using real-time PCR, the positive samples will have their entire VP2 gene sequenced for phylogenetic analyses. Spatial analyses will be conducted on the samples, in addition to determining their genetic similarity and likely patterns of evolutionary divergence. The goal is to measure cross-species epidemiology, spillover to domestic cats and dogs, and potential spillback to wildlife populations.

Comments on relevance of topic to Morris Animal Foundation:

This project is highly relevant in terms of wildlife health, and epidemiology of viral pathogens that can pass between domestic and wild animal hosts.

Comments on potential impact of project on identified field:

The impact will be large for understanding how diversity in parvoviruses evolves, and potentially the evolution of pathogenicity, as the virus is transmitted among hosts.

For Fellowship Training and First Awards, comment on candidate, mentor and training environment:

N/A

Overall assessment of scientific merit, relevance of topic, and potential impact on identified field:

Describe any animal involvement in this project including live animals, client-owned animals, laboratory animals, samples, etc.

Samples are of convenience, from vaccination campaigns of domestic dogs and cats, and other wildlife studies in and around Serengeti National Park. No additional samples will be obtained for this study.

Potential for inventions:

None

Additional pertinent comments or information:

Are any of the samples linked to clinical cases of parvovirus infection? It was suggested in panel discussion that the condition of many samples in this collection may be degraded, and that the quality of DNA from samples should be determined after extraction and before RT-PCR procedures are applied to amplify and sequence them. Further, providing additional information about how much variation is expected in the VP2 gene within each strain (e.g. how many variable sites, SNPs), to give an idea of how well the virus phylogeny may be resolved.

Reply

Are any of the samples linked to clinical cases of parvovirus infection?

We will randomly select the samples and no link to clinical cases of parvovirus infection will be favored, in order to be able to establish the prevalence of infection.

In the case of dog samples (*Canis lupus familiaris*) and cats (*Felis catus*), we will choose the available samples with spatiotemporal relationships with those from wild carnivores. However, all information available about these individuals, such as age or sex, will be collected for statistics purposes.

Wild carnivores samples were collected opportunistically from chemically immobilized wild animals and postmortem examinations. In this case, all data from clinical reports and postmortem examinations will be examined to detect clinical cases of parvovirus infection and infer statistics associations.

Prevalence of parvovirus infection is high in wild and domestic asymptomatic carnivores (Allison et al., 2013; Castanheira et al., 2014). Our preliminary studies conducted in Tanzania followed the same sampling method, and they found a percentage of positives of 10,4% in dogs and 9,1% in free-living carnivore. In this study only CPV-2a and CPV-2b were searched; consequently, we expect higher rates since we are adding CPV-2c and FPLV to the research.

It was suggested in panel discussion that the condition of many samples in this collection may be degraded, and that the quality of DNA from samples should be determined after extraction and before RT-PCR procedures are applied to amplify and sequence them.

In order to assess the quality of the purified DNA from samples, before performing real-time PCR, housekeeping gene amplification will be performed by real-time PCR. This will be carried out using the universal primer set that amplifies a fragment of 248 bp of the b-actin gene previously described (Bellis et al., 2003).

All samples to be used in this current study will be obtained from the sample bank collected and stored by the Carnivore Disease Project (CDP). Many of these samples have been used in previous genetic studies and, for example, the preliminary study mentioned above has obtained good results using these samples. Furthermore, the real-time PCR technique we have adapted in our laboratory in Spain (INIA-CISA) in the preliminary study, has successfully confirmed the presence of DNA coding for VP2 in 39 out of 213 of the samples of free-living carnivores. Moreover, our newly adapted nested PCR successfully amplified 19 sequences of 1746 bp, which were later sequenced, confirming the success of the procedure. Some of these samples

were extracted from carcasses in decomposition, in the hot and humid climate conditions of Balear Islands, which have an average of around 80% humidity and 16°C temperature (Instituto Geográfico Nacional). For these reasons we expect similar success rates in this new study conducted in Tanzania.

Further, providing additional information about how much variation is expected in the VP2 gene within each strain (e.g. how many variable sites, SNPs), to give an idea of how well the virus phylogeny may be resolved.

In this resubmission we have added the requested information.

For differencing FPLV from CPV-2 groups the residues 80, 93, 103, 232, 323, 564 and 568 are very useful. A mutation Met87Leu is needed for differencing FPLV/CPV-2 from CPV-2a/-2b/-2c. Another important residue for the molecular epidemiology of the virus is the position 426. The classification based on residue 426 of the VP2 protein is currently accepted for differencing the FPLV/CPV-2/CPV-2a (Asn), CPV-2b (Asp) and CPV-2c (Glu). Despite this classification could be controversial, this is epidemiologically useful since they represent the chronological evolution of different epidemic strains (Buonavoglia et al., 2001; Nakamura et al., 2004). In order to establish recent events of spillover and spillback between domestic dogs and free living carnivores, we believe that this classification would be useful.

Allison et al. (2015) found that the residue 300 of VP2 is highly polymorphic and mutations are necessary for host adaptation. This result is not observed in our preliminary study on free-living carnivores from Spain (Calatayud et al., manuscript in revision). We have found Glycine in all our CPV-2 sequences isolated from wolves (*Canis lupus signatus*), foxes (*Vulpes vulpes*), genets (*Genetta genetta*) and badgers (*Meles meles*); as well as in all CPV-2 selected from the Genbank, which includes species as *Canis* spp –wild and domestic–, bobcat (*Lynx rufus*), stone marten (*Martes foina*) and puma (*Puma concolor*); and Alanine in all FPLV.

We believe that the differences found between Allison et al. (2015) and our study (as well as other sequences found in the Genbank), is because we detect subtypes CPV-2b and CPV-2c, and Allison et al. worked with CPV-2a. We think that subtypes CPV-2b and CPV-2c are less host specific than CPV-2 and CPV-2a and thus they don't need mutations in residue 300. This would be addressed in the present study, in which we expect to find similar results for subtypes CPV-2b and CPV-2c.

III. Name, Institution, and email address of Principal Investigator and all co-investigators.

Principal investigator: Principal investigator: Dr. Felix Lankester,
Paul G. Allen School for Global Animal Health, Washington State University,

[REDACTED]
[REDACTED]

Co-investigator: Olga Calatayud DVM, MSc Wild Animal Health.

[REDACTED]

IV. Study Proposal:

1. Specific, Testable Hypothesis and Objectives:

The hypothesis of this study is that domestic dogs and cats play a determinant role in the epidemiology of parvovirus in wild carnivores in the Serengeti-Maasai Mara ecosystem. Domestic animals maintain the virus in the ecosystem and contribute new strains to the ecology of the virus. Thus, a close phylogenetic relationship among virus strains may be found, and wild carnivores analyzed near human settlements may show a higher prevalence of the virus.

The final objective of this study is improving measures for conservation of the African wild carnivores. These measures should be chosen in accordance to the current scenario found in Tanzania, what is our subject of study. We have marked to understand the molecular epidemiology of parvovirus in this environment, through four objectives:

- 1) Estimate the prevalence of infection in our samples and the susceptibility of different species to be infected by canine and feline parvovirus (FPLV).
- 2) Characterize and classify the strains circulating among carnivores in Tanzania to improve the current vaccination programs in domestic dogs.
- 3) Perform a phylogenetic comparison of the strains infecting wild and domestic carnivores and assess the cross-species transmission. Also compare these strains with the strains found in the Genbank to know if the global evolution of parvovirus is influencing the Tanzanian epidemiology by the introduction of exotic strains.
- 4) Evaluate the proximity to humanized areas as an influent factor of infection in wild populations.

This study aims to understand the role of domestic animals in the epidemiology of parvovirus, by investigating routes of transmission and species acting as reservoir of infection to know if parvovirus is self-sustaining in the Serengeti Maasai Mara wildlife. This knowledge may be useful to assess preventive measures such as parvovirus vaccination of domestic dogs, and the control of animal translocations across national borders. Furthermore, data obtained in this study will lead to a broader research project that will investigate the influence that mass dog vaccination has on the ecology of parvovirus in wild and domestic carnivores.

2. Justification, Significance and Literature Review:

The Serengeti Maasai Mara ecosystem is a UNESCO Biosphere Reserve due to its natural heritage and biodiversity, and it has an important touristic potential for Kenya and Tanzania development. Growing evidence points to the importance of conserving carnivores because they have a pivotal role in maintaining ecosystem dynamics through trophic cascades (Ripple and Beschta, 2004). In addition to this, some of the carnivore species inhabiting this ecosystem, such as the wild dog (*Lycaon pictus*), are classified as threatened by the IUCN (Woodroffe & Sillero-Zubiri, 2012).

The introduction of non-native fauna, such as domestic animals, has different consequences in ecosystems, and the transmission of infectious diseases to naive wild populations is an important side effect of this kind of human activity. There are published several cases where introduced infectious diseases have been involved in the decline and local extinction of native species, such as the previous rabies and distemper outbreaks in the Serengeti Maasai Mara ecosystem. The impact may be more severe when an introduced host population maintains a generalist pathogen and it spillover into less abundant native populations (Cleaveland et al., 2002). This could be the case of parvovirus in Tanzania, where domestic dogs occupied the same landscape than threatened carnivore populations (Woodroffe and Donnelly, 2011).

According to Mech et al. (2008) parvoviruses may undermine certain wild population growth. Moreover, some species among the Tanzanian carnivores, such as the cheetah (*Acinonyx jubatus*), has low genetic diversity levels (Durant et al., 2008) and could be more vulnerable to infectious agents, as has been previously reported (Acevedo-Whitehouse et al., 2003).

Parvoviruses are non-enveloped, icosahedral viruses with a small capsid and a single-stranded DNA consisting of approximately 5000 base pairs (bp). VP2 is the most abundant structural protein and it determines the raised region of the capsid, known as the 'threefold spike'. This region is highly antigenic and serves as a target for neutralizing antibodies. It determines adaptations to hosts and also characterises the emergent strains (Shackelton et al., 2005).

CPV and FPLV viruses belong to the feline subgroup of parvoviruses and are grouped together with other viruses such as mink enteritis virus, racoon parvovirus and blue fox parvovirus. CPV and FPLV have a genome homology of 98% and most differences are accumulated in the VP2 domain (Truyen et al., 1998). CPV-2 was first identified by Polymerase Chain Reaction (PCR) in a domestic dog in USA in 1978 (Appel et al., 1979), following the emergence of a previously unrecognized disease in dogs, that rapidly became pandemic. However, FPLV has been known since the 1920s (Verge & Cristoforoni, 1928), and evolutionary studies have traditionally considered that FPV is the ancestor of CPV-2. According to Truyen (1998), CPV emerged from a FPV-like virus that was most likely present in a wild carnivore species -possibly the red fox (*Vulpes vulpes*)-, thereby underlining the importance of wildlife in the epidemiology of these viruses. On the other hand, recent phylogenetic studies suggest that both viruses were derived separately from common ancestors, and evolved independently before the first description of CPV in dogs (Allison et al., 2013).

CPV and FPLV follow different epidemiologic patterns. Since the emergence of CPV-2 in the 1970s, antigenic drift has been constantly changing this parvovirus. The original 1978 virus strain was replaced worldwide by CPV-2a in 1979–1980. The 2b variant was detected in the United States in 1984 and 2c in Germany in 1996 (Decaro et al. 2008). The initial CPV-2, that was unable to infect cats, regained this ability to infect these domestic animals and other carnivores with the new antigenic subtype CPV-2a (Truyen et al. 1998). It is assumed that CPV follows an epidemic pattern since its growth rate and host range have increased. Moreover, new antigenic variants have arisen over the past three decades due to a high substitution rate of about 10^{-4} substitutions per site per year. By contrast, the FPLV follows endemic patterns in cats since it has maintained a constant population size, and varies at slower rates (Shackelton et al., 2005). These viruses are currently widespread but no molecular studies have been done in East Africa. The distribution of CPV-2 strains varies depending on the continent. While the most predominant strain in Asia is 2a (Ohshima et al., 2008), in America is 2b and 2c (Hong et al., 2007), and few studies are done in Africa. In Tunisia, for example, the three strains have been found (Touihri et al., 2009).

Despite their wide host range, little is known about either the distribution of the CPV and FPV virus clades, or the susceptibility of free-ranging animals to infection. Molecular detection in hosts other than domestic cats and dogs are usually found in artificial settings such as zoos or wildlife centres (Barlow et al., 2012). However, the few genetic studies that have studied wild carnivores suggest that parvoviruses are widely distributed in these species, and that a high level of strain variation exists (Truyen et al., 1998).

Symptoms of parvovirus infection are similar in wild and domestic carnivores and primary effect of CPV is mortality in young pups (Mech et al., 2008). Parvoviruses cannot induce mitosis and can only replicate in actively dividing cells during the S phase of the cell cycle, for this reason, cubs are more vulnerable to disease (Shackelton et al., 2005). Lymphopenia or leukopenia and hemorrhagic enteritis are the most pronounced clinical symptoms and these manifestation can vary from severe to subclinical (Hoelzer and Parrish, 2010). Maternal acquired immunity is crucial for protection during the early stages of life, and pups without this protection become vulnerable when they emerge from the den (Mech et al., 2008). However, recent studies have demonstrated that the new strain CPV-2c prompts more severe pathogenicity with higher mortality rates and even vaccinated adult dogs can develop the disease (Decaro et al., 2008). CPV-2c seems to be more infectious and to induce a greater frequency of disease than previous strains (Nakamura et al., 2001).

Transmission without direct contact may be mainly thanks to the faecal-oral route. Parvoviruses may be shed in faeces for more than six months and they are highly resistant to changes in pH, temperature, solvents and desiccation. It can persist for over seven months in the environment and can be transmitted long distances by fomites (Kerr et al., 2005; Mech et al., 2008). These characteristics enable them to spread rapidly and the emergence of new strains could represent a risk for naïve populations. An example of this is CPV-2, that has been reported to cause mortality in young wild carnivores and is suspected to have triggered declines in naïve wolf populations worldwide (Mech et al., 2008).

For these reasons, it is important to understand the molecular epidemiology of parvoviruses,

since it would allow us to detect the threatened populations at risk. Furthermore, the study of the host-parasite systems is important for the understanding of host range alteration and the evolution of virulence, which will determine the emergence of new diseases (Ikeda et al., 2002). This information is needed to determine the optimum control measures to minimize the impact of domestic animals in the ecosystem, and establish which techniques will be most effective and sustainable for protecting endangered carnivores from disease. This is one of the gaps in knowledge established by the IUCN (International Union for Conservation of Nature), Red List of Threatened Species, for the conservation of some African carnivores (Woodroffe & Sillero-Zubiri, 2012).

3. Preliminary Data:

Two preliminary studies have been carried. First, we conducted a study in Tanzania on 11 wild carnivores species and asymptomatic domestic dogs (Mwalongo et al., 2014). Blood and tissues samples were collected from 2002 in Serengeti National Park and they come from the same sample bank that we are using in this current study. We tested for CPV-2a and CPV-2b by deploying convectional PCR and both strains were detected, with hosts being jackals (*Canis aureus*), African wild dogs, lions (*Panthera leo*), dwarf mongoose (*Helogale parvula*) and domestic dog (Table 1).

	Total prevalence of CPV-2a and CPV-2b (95% CI)	Positives to CPV-2a	Positives to CPV-2b
Wild carnivores	9.1 (4.6-13.6)	2/154	12/154
Domestic dogs	10.4 (3.58-17.22)	5/77	3/77

Table 1: Results from the preliminary study in Tanzania

This preliminary study reveals the presence of CPV-2a and CPV-2b in domestic dogs and wild carnivores from Tanzania; however, further investigations are needed. These results suggest that cross species could happen but sequencing the VP2 protein and comparing the strains found in different species would allow us to investigate the evolution and transmission patterns of the virus. Furthermore, a spatial analysis would confirm the spillover from domestic to native carnivores. This information would help us to determine the importance of the contact between species and therefore, the relevance of adopting control measures. For economic limitations, this previous study did not search the CPV-2c and FPLV strains and therefore there is a gap in the knowledge about the parvovirus strains circulating in the ecosystem, that need to be studied in order to assess the correct vaccination programs.

The second preliminary study has been conducted in the Animal Health Research Centre in Spain (INIA-CISA) (Calatayud et al., manuscript in revision). It is an epidemiological research of parvovirus infection in free-ranging carnivores in Spain. We conducted real-time PCR on spleen samples from 213 wild carnivores belonging to five families. Some of these samples were extracted from carcasses in decomposition. The real-time PCR technique we have adapted in our laboratory in Spain (INIA-CISA) has successfully confirmed the presence of DNA coding for VP2 in 39 of the total samples. The newly adapted nested PCR successfully amplified the sequences of 1746 bp from 19 of these positive samples, and we expect similar success rates in this current study.

The phylogenetic study revealed a high diversity of sequences, finding 15 different nucleotide sequence types (ntST). All positive Iberian wolves and one wildcat (*Felis silvestris*) were infected by CPV-2c, while the stone martens were infected by FPLV and CPV-2b. This research supports the hypothesis that cross-species transmission of parvovirus between carnivores takes place and that parvovirus host specificity is less strict than previously believed. The prevalence of infection of parvovirus in Iberian wolves was 67.6% (C.I.=50.2–82%) and a spatial analysis revealed that the proximity to urban areas is a risk factor for wolves to get infected (Millán et al., in press). The virus would not seem to persist in wild carnivore populations without the anthropogenic influence in the Spanish scenario.

Regarding the phylogenetic analysis, several aminoacid residues of the VP2 protein have been proven to be essential for molecular epidemiology and taxonomy. In first place, for differencing FPLV from CPV-2 groups the residues 80, 93, 103, 232, 323, 564 and 568 are very useful

(Allison et al., 2015). A mutation Met87Leu is needed for differencing FLPV/CPV-2 from CPV-2a/-2b/-2c. Another important residue for the molecular epidemiology of the virus is the position 426. The classification based on residue 426 of the VP2 is currently accepted for differencing the FPLV/CPV-2/2a (Asn), CPV-2b (Asp) and CPV-2c (Glu). Despite this classification could be controversial, this is epidemiologically useful since they represent the chronological evolution of different epidemic strains (Buonavoglia et al., 2001; Nakamura et al., 2004). In order to establish recent events of spillover introduction from domestic dogs to wild carnivores we believe that this classification would be useful.

Allison et al. (2015) found that the residue 300 of VP2 is highly polymorphic and mutations are necessary for host adaptation. This result is not observed in our preliminary study on free-living carnivores from Spain (Calatayud et al., manuscript in revision). We have found Glycine in all our CPV-2 sequences isolated from wolves, foxes, genets and badgers; as well as in all CPV-2 selected from the Genbank, which includes species as *Canis* spp –wild and domestic–, bobcat, stone marten and puma; and Alanine in all FPLV.

We believe that the differences found between Allison et al. (2015) and our study (as well as other sequences found in the Genbank), are because we detect subtypes CPV-2b and CPV-2c, and Allison et al. worked with CPV-2a. We think that subtypes CPV-2b and CPV-2c are less host-specific than CPV-2 and CPV-2a and thus they don't need mutations in residue 300. This question would be addressed in the present study, where we expect to find similar results for subtypes CPV-2b and CPV-2c.

4. Experimental Methods and Design:

To achieve our main goal we will conduct 1) investigation of the presence of CPV-2 and FPLV in free-living and domestic carnivores in Tanzania by real-time PCR; 2) characterization of the parvovirus strains by sequencing the complete VP2 gene and further inferring the phylogenetic associations of the obtained isolates; 3) Spatial analysis of the prevalence.

Main outcomes will be represented as:

- 1) Prevalence of subgroup of parvoviruses in both wild and domestic carnivores, to know the susceptibility of these animals.
- 2) Classification of the subgroups of parvoviruses (FPLV, CPV-2, 2a, 2b, 2c) infecting different carnivore species and the construction of a phylogenetic tree.
- 3) Distribution map to visualize the distribution of the isolates and their relationship among humanized areas.

4.1. Investigation of the presence of CPV-2 and FPLV

4.1.1. Stool samples of free-living carnivores.

Wild carnivores samples used in this study were collected opportunistically from chemically immobilized wild animals and postmortem examinations performed in the Serengeti National Park between 2002 and 2009. The samples were collected by the Carnivore Disease Project (CDP) under the Tanzanian Wildlife Research Institute (TAWIRI) permission.

We will select 150 samples available from the CDP belonging the following species: lions, jackals, African wild dogs, dwarf mongooses, cheetah, hyenas (*Crocuta crocuta*), leopards (*Panthera pardus*), civets (*Civettictis civetta*), aard wolves (*Proteles cristata*), bat-eared foxes (*Otocyon megalotis*), and servals (*Leptailurus serval*). These samples will be selected randomly to be able to establish the prevalence of infection. However, data from clinical reports and postmortem examinations will be examined to detect clinical cases of parvovirus infection and infer statistic associations.

4.1.2 Stool samples of domestic dogs.

Since 2003 the Serengeti Health Initiative (SHI) has been carrying out mass dog vaccination campaigns in districts surrounding the Serengeti National Park. During these campaigns domestic dogs and cats are brought to the central village point, where they receive a health check and a sub-cutaneous inoculation against rabies, distemper and parvovirus. In addition, a 10 ml blood sample is collected from the cephalic vein of all dogs that appear to be under one year of age. These samples were frozen and are available for the current study. We will choose 150 of these available samples with spatiotemporal relationships with those from wild carnivores. They will be randomly selected in each area to infer the prevalence of infection, and

no link to clinical cases of parvovirus infection will be favored. All information available about these individuals, such as age or sex, will be collected for statistic purposes.

4.1.3. Real-time PCR detection of CPV-2 and FPLV in all samples

This step will be done in the Nelson Mandela African Institution of Science and Technology laboratory in Arusha, Tanzania. Total DNA of the samples will be isolated by the method of spin columns, which it is routinely used in our lab. In order to assess the quality of the purified DNA, housekeeping gene amplification will be performed by real time PCR. This will be carried out using the universal primer set that amplifies a fragment of 248 bp of the b-actin gene previously described (Bellis et al., 2003).

Once validated the quality of the DNA, a real time PCR based on Taqman probes will be performed in all the samples (Decaro et al., 2007). This real time PCR is capable to detect with a high sensibility and specificity all positives to CPV-2, 2a, 2b and 2c, plus all positives to FPLV. A vaccine will be used as positive control (Nobivac® puppy).

4.2. Characterization and sequencing of the strains

This step will be done in the INIA-CISA (Animal Health Research Institute, Madrid, Spain), following the same steps than in the preliminary study conducted in Spanish wild carnivores:

4.2.1. Characterization.

We will characterize the VP2 coding region of all positive samples. This region will be amplified by two nested PCR using two sets of primers as described previously, and we will use a vaccine as positive control (Nobivac® puppy).

4.2.2. Sequencing.

We will sequence the amplicons obtained from the PCRs described above, using seven internal primers designed in previous studies. Due to the fact that we are trying to amplify long fragments of naturally infected carnivores without clinical signs -and probably with low copies of virus in the target tissues-, we expect that some positive samples could be negative or weak positive. In the last case, in order to obtain clear sequences, we will clone these amplicons in *Escherichia coli* vectors using a commercial kit.

4.2.3. Phylogenetic analysis

This analysis comprises the following steps:

4.2.3.1. Alignment of the sequences with MEGA 6 software to obtain a consensus sequence for each positive sample, plus translation of the nucleotide sequences into amino acid.

4.2.3.2. Blast search in the Genbank of the consensus sequences. All sequences of the VP2 genes available in the database will be included in the study.

4.2.3.3. Phylogenetic analysis with MEGA 6 Software using different algorithms (Neighbor-Joining and Maximum Likelihood), based on p-distances (nucleotide and amino acid).

The main outcome of this step will be to describe for each obtained sequence: a) the identification of the parvovirus (FPLV/CPV-2) and CPV-2 subtype, b) the phylogenetic relationships within the different subtypes.

4.3. Spatial analysis of the prevalences

The main outcome will be to explore the statistic relationship among the strains and other variables, such as host species (domestic vs. wild animals), geographical distribution, age, gender, etc. We will use ArcGIS Software for spatial analysis.

5. Timeline:

The project is expected to last one year, from June 2016 to July 2017. Each column represents approximately a trimester.

Tasks	2016		2017	
Sample selection and preparation of laboratorial works				
DNA extraction and real time PCR screening of 300 samples (wild carnivores and domestic dogs).				
Amplification of VP2 gene in positive samples				
Sequencing VP2/sequence assembly/phylogenetic study				
Epidemiological study				
Final report				

V. Animal Involvement Justification:

Morris Animal Foundation Animal Involvement Justification

Morris Animal Foundation (MAF) is dedicated to funding scientifically sound, relevant and humane studies that specifically address the health and well-being of animals. All studies receiving funding must follow MAF's Health Study Policy for Animals Involved in Research (adopted October 18, 2008), which was written to ensure that each and every animal involved in a MAF funded health study receives excellent, compassionate care throughout the study. MAF shall not fund health studies which require euthanasia as an endpoint or the induction of disease or injury, unless the nature of the disease or condition to be studied is of such significance for improving animal health that such means are justified, and that meaningful information can be obtained in no other way. Furthermore, MAF will not fund any study that induces or allows pain or distress unless such pain or distress can be controlled by appropriate anesthetic, analgesic, tranquilizing drugs, or nursing care. [Click here](#) for the full Health Study Policy.

A. If this study does not involve live animals please indicate here by N/A: N/A

B. Does this study involve biological samples, tissues, etc.? Yes

If yes, describe in detail what samples will be used and where & how they will be (or were) acquired. Note: Morris Animal Foundation reserves the right to request a copy of the Institutional Animal Care and Use Committee (IACUC) application/approval and other relevant applications/approvals (e.g., wildlife permit) covering the original collection of samples, including archived samples. MAF reserves the right to request IACUC (or equivalent) review and approval for any Foundation study **regardless of the Institution's requirements**. This would include the use of archived samples as well as clinical trials.

This study involves blood and spleen samples, which have been previously collected. We distinguish samples collected from free-living and domestic animals below:

Stool samples of free-living carnivores.

Wild carnivores samples used in this study were collected opportunistically from chemically immobilized wild animals (blood samples) and postmortem examinations (spleen samples) performed in the Serengeti National Park between 2002 and 2009. The samples were collected by the Carnivore Disease Project (CDP) under the Tanzanian Wildlife Research Institute (TAWIRI) permission.

We will select 150 samples available from the CDP belonging the following species: lions (*Panthera leo*),

Jackals (*Canis aureus*), African wild dogs (*Lycaon pictus*), dwarf mongooses (*Helogale parvula*), cheetah (*Acinonyx jubatus*), hyenas (*Crocuta crocuta*), leopards (*Panthera pardus*), civets (*Civettictis civetta*), aard wolves (*Proteles cristata*), bat-eared foxes (*Otocyon megalotis*), and servals (*Leptailurus serval*). These samples will be selected randomly to be able to establish the prevalence of infection.

Stool samples of domestic dogs.

Since 2003 the Serengeti Health Initiative (SHI) has been carrying out mass dog vaccination campaigns in districts surrounding the Serengeti National Park. During these campaigns domestic dogs and cats are brought to the central village point, where they receive a health check and a sub-cutaneous inoculation against rabies, distemper and parvovirus. In addition, a 10 ml blood sample is collected from the cephalic vein of all dogs that appear to be under one year of age. These samples were frozen and are available for the current study. We will choose 150 of these available samples with spatiotemporal relationships with those from wild carnivores. They will be randomly selected in each area to infer the prevalence of infection, and no link to clinical cases of parvovirus infection will be favored.

C. If this study involves live animals, succinctly address the following: (please restate the questions and directives). N/A

1. What species will be studied? N/A
2. State the status of your IACUC application/approval. All recipients of MAF funding will be required to submit the entire IACUC protocol and document. A copy of the IACUC approval should not be included with the application, but it is required before funding can be awarded.
3. List the USDA category for pain and distress (B, C, D, E): N/A

Note: Any study beyond category C will require review by MAF's Animal Welfare Advisory Board (AWAB). In general MAF does not fund studies beyond category C (category D studies will only be considered if they conform with MAF's Health Study Policy, category E studies will not be considered).

4. Does this proposal involve client-owned animals? N/A

If yes, the protocol for client-owned animals must be approved by the appropriate peer review committee before the project is funded. *If this proposal involves client-owned animals, an informed client consent form must be submitted with this proposal. For a suggested list of items to be considered in an informed client consent form, [click here](#).*

5. Explain how animals will be acquired (e.g., client-owned, USDA licensed breeder, institutional "herds" or "colonies") and verify that the animals are suitable for the study (e.g., have no physiologic, physical or pharmacologic issues that would interfere with results) N/A

6. How many animals will be used? N/A

- a. Summarize numerical justification

7. Does this study induce disease, injury, pain or distress in animals? Note: any study requiring the induction of disease, injury, pain, or distress will have an additional evaluation by MAF's AWAB. No

If yes,

- a. Defend the necessity of experimental design
- b. Explain how pain and/or distress will be controlled
- c. Justify that no alternative, including clinical studies, can be used to accomplish study objectives and the disease/condition to be studied is of such significance for improving the health of the species.

8. Explain the environment and housing conditions (quality of life) in which the animals will live (address species-appropriate exercise, enrichment, socialization, veterinary care, etc.) N/A

9. What will happen to the animals upon completion of the study? N/A

If adoption, explain the adoption process. Provide assurance that whenever possible and when in the animal's best interest, investigators shall make companion animals available for adoption at the end of the study or return the animals to the owner/responsible agency in an environment that promotes animal welfare and excellent quality of life.

10. If euthanasia, provide the following additional information (note: any study requiring euthanasia as an endpoint will have an additional evaluation by a MAF's AWAB. N/A

- i. Total number that will be euthanized and justification for numbers

- ii. Method of euthanasia

- iii. Justification that no alternatives can be used to accomplish study goal(s) and that the disease/condition to be studied is of such significance for improving the health of the species that a terminal endpoint is deemed necessary.

- iv. Reason for euthanasia in lay language (this wording may be shared with staff,

donors and media)

- v. Provide objective criteria for determining when euthanasia is appropriate or necessary (note: Morris Animal Foundation wants assurance that an animal will not be allowed to suffer and that monitoring for pain and suffering is adequate)

Note: Morris Animal Foundation does not consider the use of CO₂ alone to be an appropriate method of euthanasia

Please note:

1. If an animal is used in an invasive study, MAF may require that a guarantee is provided, through principal investigator and institutional signatures that the animal will not participate in any future invasive study or procedure
2. MAF does not allow inclusion of ancillary data in MAF funded research that includes animal use protocols not in agreement with our Health Study Policy, even if it is obtained using other funding sources.
3. Morris Animal Foundation considers euthanasia acceptable when an animal develops unanticipated illness or injury that results in pain and suffering that cannot be alleviated with standard veterinary interventions.

VI. Recombinant DNA/Biohazards: N/A

VII. Facilities and Equipment.

Facilities at the Nelson Mandela African Institution of Science and Technology laboratory in Arusha, Tanzania.

The laboratory has the full equipment for real time PCR detection: real time thermocycler, DNA extraction materials, cabins, centrifuges...

Facilities at the Animal Health Research Centre (INIA-CISA).

The laboratory has the full equipment for PCR detection, sequencing and cloning: thermocycler, cabins, centrifuges, automatic sequencer, microbiological lab...

VIII. Cited References

- Acevedo-Whitehouse, K., Gulland, F., Greig, D., Amos, W., 2003. Inbreeding: Disease susceptibility in California sea lions. *Nature* 422, 35.
- Allison, A.B., Kohler, D.J., Fox, K. a, Brown, J.D., Gerhold, R.W., Shearn-Bochsler, V.I., Dubovi, E.J., Parrish, C.R., Holmes, E.C., 2013. Frequent cross-species transmission of parvoviruses among diverse carnivore hosts. *J Virol* 87, 2342–7.
- Allison, A.B., Organtini, L.J., Zhang, S., Hafenstein, S.L., Holmes, E.C., Parrish, C.R., 2015. Single mutations in the VP2 300 loop region of the three-fold spike of the carnivore parvovirus capsid can determine host range. *J Virol* p. JVI.02636-15.
- Appel, M., Scott, F., Carmichael, L., 1979. Isolation and immunisation studies of a canine parvo-like virus from dogs with haemorrhagic enteritis. *Vet Rec* 105, 156–159.
- Barlow, A.M., Schock, A., Bradshaw, J., Mullineaux, E., Dastjerdi, A., Everest, D.J., McGowan, S., Steinbach, F., Cowen, S., 2012. Parvovirus enteritis in Eurasian badgers (*Meles meles*). *Vet Rec* 170, 416.
- Bellis, C., Ashton, K., Freney, L., Blair, B., Griffiths, L., 2003. A molecular genetic approach for forensic animal species identification. *Forensic Sci Int* 134(2-3):99-108.
- Buonavoglia, C., Martella, V., Pratelli, a, Tempesta, M., Cavalli, a, Buonavoglia, D., Bozzo, G., Elia, G., Decaro, N., Carmichael, L., 2001. Evidence for evolution of canine parvovirus type 2 in Italy. *J Gen Virol* 82, 3021–5.
- Castanheira, P., Duarte, A., Gil, S., Cartaxeiro, C., Malta, M., Vieira, S., Tavares, L., 2014. Molecular and serological surveillance of canine enteric viruses in stray dogs from Vila do Maio, Cape Verde. *BMC Vet Res* 10, 91.
- Cleaveland, S., Hess, G.R., Dobson, A., Laurenson, M.K., McCallum, H.I., Roberts, M., Woodroffe, R., 2002. The role of pathogens in biological conservation. *The Ecology of Wildlife Diseases* 139–150, Oxford, UK: Oxford University Press.
- Decaro, N., Desario, C., Addie, D.D., Martella, V., Vieira, M.J., Elia, G., Zicola, A., Davis, C., Thompson, G., Thiry, E., Truyen, U., Buonavoglia, C., 2007. Molecular epidemiology of canine parvovirus, Europe. *Emerg Infect Dis* 13, 12–14.
- Decaro, N., Desario, C., Elia, G., Martella, V., Mari, V., Lavazza, A., Nardi, M., Buonavoglia, C., 2008. Evidence for immunisation failure in vaccinated adult dogs infected with canine parvovirus type 2c. *New Microbiol* 31, 125–30.
- Hong, C., Decaro, N., Desario, C., Tanner, P., Pardo, M.C., Sanchez, S., Buonavoglia, C., Saliki, J.T., 2007. Occurrence of Canine Parvovirus Type 2c in the United States. *J Vet Diagnostic Investig* 19, 535–539.
- Hoelzer, K., Parrish, C.R., 2010. The emergence of parvoviruses of carnivores. *Vet Res* 41, 39.
- Ikeda, Y., Nakamura, K., Miyazawa, T., Takahashi, E., Mochizuki, M., 2002. Feline host range of canine parvovirus: recent emergence of new antigenic types in cats. *Emerg Infect Dis* 8, 341–6.
- Mech, L.D., Goyal, S.M., Paul, W.J., Newton, W.E., 2008. Demographic effects of canine

- parvovirus on a free-ranging wolf population over 30 years. *J Wildl Dis* 44, 824–36.
- Millán, J., López-Bao, J.V., García, E.J., Oleaga, Á., Llaneza, L., Palacios, V., Rodríguez, A., Torre, A. de la, Dubovi, E.J., **Esperón, F.**, n.d. Patterns of exposure of Iberian wolves (*Canis lupus*) to canine viruses in human-dominated landscapes. *Ecohealth Press ECH-15-0042* (in press).
- Mwalongo, O., Shahada, F., Bigambo, M., Gwakisa, P., **Lankester, F.**, 2014. Prevalence of Canine Parvovirus in Domestic Dogs around Serengeti National Park (Tanzania). *Int J Sci Res* 3, 1864–1868.
- Nakamura, K., Sakamoto, M., Ikeda, Y., Kawakami, K., Miyazawa, T., Takahashi, E., Mikami, T., 2001. Pathogenic Potential of Canine Parvovirus Types 2a and 2c in Domestic Cats Pathogenic Potential of Canine Parvovirus Types 2a and 2c in Domestic Cats. *Clin Diagn Lab Immunol* 8, 663–668.
- Nakamura, M., Tohya, Y., Miyazawa, T., Mochizuki, M., Phung, H., Nguyen, N., Huynh, L., Nguyen, L., Nguyen, P., Nguyen, P., Nguyen, N., Akashi, H., 2004. A novel antigenic variant of Canine parvovirus from a Vietnamese dog. *Arch Virol* 149, 2261–9.
- Ohshima, T., Hisaka, M., Kawakami, K., Kishi, M., Tohya, Y., Mochizuki, M., 2008. Chronological Analysis of Canine Parvovirus Type 2 Isolates in Japan. *J Vet Med Sci* 70, 769–775.
- Reed, a P., Jones, E. V, Miller, T.J., 1988. Nucleotide sequence and genome organization of canine parvovirus. *J Virol* 62, 266–76.
- Ripple, W.J., Beschta, R.L., 2004. Wolves and the Ecology of Fear: Can Predation Risk Structure Ecosystems? *Bioscience* 54, 755.
- Shackelton, L. a, Parrish, C.R., Truyen, U., Holmes, E.C., 2005. High rate of viral evolution associated with the emergence of carnivore parvovirus. *Proc Natl Acad Sci USA* 102, 379–84.
- Touihri, L., Bouzid, I., Daoud, R., Desario, C., Goulli, A.F. El, Decaro, N., Ghorbel, A., Buonavoglia, C., Bahloul, C., 2009. Molecular characterization of canine parvovirus-2 variants circulating in Tunisia - Springer. *Virus Genes* 38, 248–58.
- Truyen, U., Muller, T., Heidrich, R., Tackmann, K., Carmichael, L.E., 1998. Survey on viral pathogens in wild red foxes (*Vulpes vulpes*) in Germany with emphasis on parvoviruses and analysis of a DNA sequence from a red fox parvovirus. *Epidemiol Infect* 121, 433–440.
- Truyen, U.W.E., Evermann, J.F., Vieler, E., Parrish, C.R., 1996. Evolution of Canine Parvovirus Involved Loss and Gain of Feline Host Range 189, 186–189.
- Woodroffe, R., Donnelly, C. a., 2011. Risk of contact between endangered African wild dogs *Lycaon pictus* and domestic dogs: opportunities for pathogen transmission. *J Appl Ecol* 48, 1345–1354.

IX. Budget

Category	
<u>Personnel:</u>	
1. Co-investigator (Olga Calatayud)	██████
Total Salaries	██████
<u>Supplies & Expenses:</u>	
1. PCR reagents and plastics for real time detection	██████
2. Service of molecular diagnostics	██████
3. Transport, accommodation and meals in Tanzania.	██████
4. Courier and permits for sample transport.	██████
Total Supplies & Expenses:	██████
Subtotal of All Categories:	\$ 10000
Maximum of 8% - Indirect Costs:**	\$ 800
Grand Total Requested from MAF:	\$ 10800

X. Itemized Budget Justification

Salary Co-Investigator (Olga Calatayud): This will afford the salary of two months of work, one of them at the Nelson Mandela African Institution of Science and Technology laboratory in Arusha, Tanzania; and the other month at the Animal Health Research Centre, in Madrid, Spain.

Service of molecular characterization: The samples will be analyzed in the Animal Health Research Center (INIA-CISA), as an external service. The responsible of the analysis is Dr. Fernando Esperón, which participates in this proposal as resource personnel.

Transport, accommodation and meals in Tanzania: These costs will be used for a stay of a month of the Co-Investigator Olga Calatayud at the Nelson Mandela African Institution of Science and Technology laboratory in Arusha, Tanzania. During this stay she will collaborate in DNA extraction and perform the real-time PCR for parvovirus detection. She has full expertise on parvovirus detection and characterization, since her MSc research project was entitled: "Molecular characterization of parvovirus infection in free ranging carnivores in Spain".

Courier and permits for sample transport: Necessary to transport the samples from the Nelson Mandela African Institution of Science and Technology (Tanzania) laboratory to the Animal Health Research Centre (Spain).

XI. Other Support: N/A.

XII. Prior MAF Support during the last three years: N/A.

XIII. Biographical Data (two-page limit for each individual):

Full name:	Dr. Felix Lankester
Present position:	Clinical Assistant Professor – Paul G. Allen School for Global Animal Health, Washington State University
	Post-Graduate Supervisor – Nelson Mandela African Institute of Science and Technology (NM-AIST)
	Regional Representative – Global Animal Health Tanzania
	Director, Serengeti Health Initiative
	Veterinary Director for Pandrillus Foundation

Education/Training:

2010 – present. PhD candidate - University of Glasgow, UK. Title: Malignant catarrhal fever: a field-based vaccine trial and an economic impact assessment of the disease and its control on pastoralist livelihoods in Tanzania.

2002 – 2003. Master of Science in Wild Animal Health - Institute of Zoology & Royal College of Veterinary Surgeons, London University, UK; graduated with merit

1990 – 1995. Bachelor of Veterinary Science - Liverpool University, UK

Previous positions:

2009 - 2012	Director of Tanzanian Programs – Lincoln Park Zoo
2006 - 2009	Country Director of Pandrillus Foundation – Cameroon
2004 - 2009	Project Director and Head Veterinarian of the Limbe Wildlife Centre (LWC)
2003 - 2004	Consultant to the Mediae Production Company, Nairobi, Kenya
2004 -2004	Associate Producer for ‘Born to Run’ (Sky Sports)
2002 - 2003	Two phases of volunteering as a veterinary surgeon for The Orang-utan Foundation, Kalimantan, Borneo
1997 - 2001	Assistant Producer for Granada Wild (Partridge Films), Bristol, UK
1995 - 2002	Veterinary surgeon of mixed and small animal practices in SW England

Honours/distinctions/membership of societies, institutions, committees:

2012-present	International Society for Veterinary Epidemiology and Economics Bursary Award
2010-present	Inspector of the Pan African Sanctuaries Alliance
2009-present	Member of Tanzania Veterinary Association
1995-present	Member of Royal College of Veterinary Surgeons

Selected Peer Reviewed Publications (four last years):

- Mwalongo, O., Shahada, F., Bigambo, M., Gwakisa, P. & **Lankester, F.** (2014) Prevalence of canine parvovirus in domestic dogs around Serengeti National Park (Tanzania). *International Journal of Science and Research*. 3 (10), 1864 - 1868
- Cleaveland, S., Hampson, K., Lembo, T., Townsend, S. and **Lankester, F.** (2014). Role of dog sterilisation and vaccination in rabies control programs. *Veterinary Record*. 2014 175: 409-410. doi: 10.1136/vr.g6352
- **Lankester, F.**, Hampson, K., Lembo, T., Taylor, L., Palmer, G. & Cleaveland, S. (2014). Implementing Pasteur’s vision for rabies elimination. *Science* 345 (6204), 1562 – 1564.
- Cleaveland, S., **Lankester, F.**, Townsend, S., Lembo, T. and Hampson, K. (2014). Rabies control and elimination: a test case for One Health. *Veterinary Record*. One Health Feature, 188-193. doi: 10.1136/vr.g4996
- Bartley, K., Deane, D., Percival, A., Dry, I., Grant, D., Inglis, N., Mclean, K., Manson, E., Imrie, L., Haig, D., **Lankester, F.** and Russell, G. (2014). Identification of immuno-reactive capsid proteins of malignant catarrhal fever viruses. *Journal of Veterinary Microbiology*. 173 (1-2), 17-26
- Cleaveland, S., Beyer, H., Hampson, K., Haydon, D., **Lankester, F.**, Lembo, T. *et al.*, (2014), ‘The changing landscape of rabies epidemiology and control’, *Onderstepoort Journal of Veterinary Research* 81(2), Art. #731, 8 pages. <http://dx.doi.org/10.4102/ojvr.v81i2.731>

- Cleaveland, S., Beyer, H., Hampson, K., Haydon, D., **Lankester, F.**, Lembo, T., Meslin, F., Morders, M., Mtema, Z., Sambo, M., Townsend, S. (In press). The changing landscape of rabies epidemiology and control, Onderstepoort Journal of Veterinary Research
- Prado-Martinez, J... **Lankester, F.J.**...and Marques-Bonet, T. (2013) Great ape genetic diversity and population history. *Nature*. 499, 471–475
- Lyons S, Sharp C, LeBreton M, Djoko CF, Kiyang JA, **Lankester F.**, Bibila, T.G., Fair J., Wolfe N. and Simmonds P. (2012) Species Association of Hepatitis B Virus (HBV) in Non-Human Apes; Evidence for Recombination between Gorilla and Chimpanzee Variants. *PLoS ONE* 7(3): e33430. doi:10.1371/journal.pone.0033430
- Cleaveland S., Keyyu J., Auty H., Lembo T., Hampson K., **Lankester F.J.** and Kazwala R. (2012). Domestic animal health programs to protect wildlife. *Animal Health and Biodiversity – Preparing for the Future*. OIE Booklet on the OIE Global Wildlife Conference, Paris
- Köndgen S, Leider M, **Lankester F**, Bethe A, Lübke-Becker A, Leendertz, F.H. and Ewers, C. (2011). *Pasteurella Multocida* Involved in Respiratory Disease of Wild Chimpanzees. *PLoS ONE* 6(9): e24236. doi:10.1371/journal.pone.0024236
- Wevers D, Metzger S, Babweteera F, Bieberbach M, Boesch C, Cameron K, Couacy-Hymann E, Cranfield M, Gray M, Harris LA, Head J, Jeffery K, Knauf S, **Lankester F**, Leendertz SA, Lonsdorf E, Mugisha L, Nitsche A, Reed P, Robbins M, Travis DA, Zommers Z, Leendertz FH, Ehlers B. (2011) Novel adenoviruses in wild primates: a high level of genetic diversity and evidence of zoonotic transmissions. *J Virol*. 85 (20): 10774-84. Epub 2011. PMID: 21835802
- Gonder, M.K., Locatelli, S., Ghobrial, L., Mitchell, M., Kujawski, J.T., **Lankester F.J.**, and Tishkoff, S. (2010) Evidence from Cameroon reveals differences in the genetic structure and histories of chimpanzee populations. In: *PNAS*, 108 (12) 4766-4771
- **Lankester, F.**, Anyam Kiyang, J., Bailey, W and Unwin, S.J. (2010) *Dientaomeba fragilis*: initial evidence of pathogenicity in the western-lowland gorilla (*Gorilla gorilla gorilla*). In: *Journal of Zoo and Wildlife Medicine*. 41(2): 350-352.
- Ghobrial, L., **Lankester, F.J.**, Kiyang, A.J., Akih, E.A., de Vries, S., Fotso, R., Gadsby, E.L., Jenkins, P.D. and Gonder, M. K. (2010) Tracing the origins of rescued chimpanzees reveals widespread chimpanzee hunting in Cameroon. In: *BMC Ecology* 10:2
- Leendertz, F., Deckers, M., Schempp, W., **Lankester, F.**, Boesch, C., Mugisha, L., Dolan, A., Gatherer, D., McGeoch, D. and Ehlers, B. (2009) Novel cytomegaloviruses in free-ranging and captive great apes: phylogenetic evidence for bidirectional horizontal transmission. In: *J Gen Virol* 90, 2386-2394.
- **Lankester, F.**, Matz-Rensing, K., Kiyang, J., Jensen, S., Weiss, S. And Leendertz, F. (2008) Fatal ulcerative colitis in a western-lowland gorilla (*Gorilla gorilla gorilla*). In: *J. Med. Primatol* 37: 297-302
- Thalmann, O., Fischer, A., **Lankester, F.**, Paabo, S. and Vigilant, L. (2007) The complex evolutionary history of gorillas: insights from genomic data. In: *Mol. Biol. Evol.* 24(1):146-158
- ***Lankester, F.**, ***Leendertz, F.** and Guislain, P. (2006) Anthrax in Western and Central African great apes. In: *American Journal of Primatology*. 68:928-933 (*authors contributed equally)
- **Lankester, F.** and Divers, S. (2005) Phallic prolapse and subsequent amputation in a Nile crocodile (*Crocodylus niloticus*). In: *Journal of Zoo and Wildlife Medicine*.
- **Lankester, F.** (2004) A probable case of fatal hyper-infection syndrome associated with nematodiasis in a wild orang-utan (*Pongo pygmaeus*). In: *World Association of Wildlife Veterinarians*. 14 (55): 3–6.

Full name:	Olga calatayud
Present position:	PhD candidate
Present work address:	N/A
Role on project	Resource personnel / molecular analysis

Education/Training:

2007. Bachelor of Veterinary Medicine (University Cardenal Herrera-CEU, Valencia, Spain).

2013-2014. Master degree in "Wild Animal Health" at the Royal Veterinary College/ Zoological Society of London, UK. MSc Research Project entitled: "Molecular characterization of parvovirus infection in free ranging carnivores in Spain".

Previous positions:

2015	Veterinarian in the small animal clinic Drs. Bustillo, Valencia, Spain
2014	Researcher in the Animal Health Research Center CISA-INIA, Madrid, Spain.
2013	Veterinarian in the small animal clinic <i>Albert</i> , Picassent, Spain.
2012	Veterinarian at Bylakuppe Rabies and Disease Control Programme, (<i>Vets Beyond Borders Australia NGO</i>). Bylakuppe, India.
2011	Veterinarian in The Thai Society for the Conservation of Wild Animals, Thailand.
2011	Veterinarian in charge of The Society for the Prevention of Cruelty to Animals of Paterna, Valencia, Spain.
2009-2010	Veterinarian in <i>Sanaga-Yong Chimpanzee Rescue Center</i> , Cameroon.
2008-2009	Veterinarian in the Small Animals Hospital, <i>Hospital Veterinario Benicarló</i> , Castellón, Spain.
2008	Animal keeper at the Zoo of primates "La Vallée des Singes", Poitiers, France.
2006-2007	Practices at Valencia Zoo, Spain.
2005-2006	Practices at the Wildlife Recovery Center <i>La Granja</i> , Valencia, Spain.
2005	Practices at the Zoo <i>Selwo-Aventura</i> , Málaga, Spain.

Membership of societies:

2008-present: Member of College of Veterinary Surgeons of Valencia, Spain.

Full name:	Fernando Esperón Fajardo
Present position:	Responsible of the Unit of Diagnostic of Wildlife Diseases, Epidemiology and Environmental Group (EySA), (CISA-INIA)
Present work address:	Animal Health Research Centre (CISA-INIA), Ctra. Algete a El Casar s/n, 28130 Valdeolmos, Madrid, Spain.
Role on project	Resource personnel/ molecular analysis

Education/Training:

2005. PhD degree. Department of Animal Health, Faculty of Veterinary Medicine (University Complutense of Madrid, Spain). Thesis entitled: “Environmental pollutants in stranded odontocetes from Canary Archipelago. Health implications”.

2003. Degree of Master on Research. Department of Animal Health, Faculty of Veterinary Medicine (University Complutense of Madrid, Spain).

1999. Bachelor of Veterinary Medicine (University Complutense of Madrid, Spain).

Previous positions:

2008 – present	Responsible of the Unit of Diagnostic of Wildlife Diseases, Epidemiology and Environmental Group (EySA), (CISA-INIA)
2006 - 2008	University graduate position (TRAGSA), Animal Health Research Centre (CISA-INIA).
2005-2006	University graduate position, Department of Animal Health, Faculty of Veterinary Medicine (University Complutense of Madrid)
2001-2005	Predoctoral Fellowship, Animal Health Research Centre (CISA-INIA).
2001-2003	Chief Executive Officer and founding partner, Sil-Ex, SLL (Private laboratory, diagnostics exotic and wildlife).
1999-2001	Small Companion Animals, DVM.

Honours/distinctions/membership of societies, institutions, committees:

2014-present	Member of Wildlife Diseases Association (WDA) and regional sections: European Wildlife Diseases Association (EWDA) and Latin American Wildlife Diseases Association (LAWDA).
2005-present	Teaching contributor on practices classes, Department of Pharmacology, Faculty of Veterinary Medicine (University Complutense of Madrid).
2002-2003	Honorific Contributor, Department of Compared Anatomy and Morphology, Faculty of Veterinary Medicine (University Complutense of Madrid).
1997-2000.	Cofounder and President of the Veterinary Association for the Assistance of Exotic Pets and Wildlife (AVAFES-Madrid).

Selected Peer Reviewed Publications (four last years):

- 2015. Javier Millán, José Vicente López-Bao, Emilio J. García, Álvaro Oleaga, Luis Llaneza, Vicente Palacios, Alejandro Rodríguez, Ana de la Torre, Edward J. Dubovi, Fernando Esperón. Patterns of exposure of Iberian wolves (*Canis lupus*) to canine viruses in human-dominated landscapes. Ecohealth. In press. ECH-15-0042.
- 2015. Millán J., López-Roig, M., Delicado, V., Serra-Cobo, J., Esperón F. 2014. Widespread infection with hemotropic mycoplasmas in bats in Spain, including a hemoplasma highly related to *Candidatus Mycoplasma hemohominis*. Comp. Immunol. Microbiol. Infect. Dis.; 39:9-12.
- 2014. Esperón F., Vázquez, B., Sánchez, A., Fernández-Pinero, J., Yuste, M., Neves, E., Nogal, V., and Maria Jesus Muñoz. 2014. *Seroprevalence of paramyxoviruses in synanthropic and semi-free range birds*. Avian Diseases 58(2):306-8.

- 2014. Sacristán C, Esperón F, Herrera-León S, Iglesias I, Neves E, Nogal V, Muñoz MJ, de la Torre A. *Virulence genes, antibiotic resistance and integrons in Escherichia coli strains isolated from synanthropic birds from Spain*. Avian Pathol. 2014;43(2):172-5.
- 2013. Cabello J, **Esperón F**, Napolitano C, Hidalgo E, Dávila JA, Millan J. (2013). *Molecular identification of a novel gammaherpesvirus in the endangered Darwin's fox (Lycalopex fulvipes)*. J Gen Virol. 94, 2745–2749.
- 2013. **Esperón F**, Martín MP, Lopes F, Orejas P, Carrero L, Muñoz MJ, Alonso R. 2013. *Gongylonema sp. infection in the scops owl (Otus scops)*. Parasitology International, 62:502-504.
- 2013. Rubio-Guerri, C.; Melero, M.; **Esperón, F.**; Bellière, E.N.; Arbelo, M; Crespo, J.L.; Sierra, E.; García-Párraga, D.; Sánchez-Vizcaíno, J.M. 2013. *Unusual striped dolphin mass mortality episode related to cetacean morbillivirus in the Spanish Mediterranean sea*. BMC Veterinary Research 2013, 9:106.
- 2013. Rubio-Guerri, C.; Melero, M.; Rivera-Arroyo, B.; Bellière, E.N.; Crespo, J.L.; García-Párraga, D.; **Esperón, F.**; Sánchez-Vizcaíno, J.M. *Simultaneous diagnosis of Cetacean morbillivirus infection in dolphins stranded in the Spanish Mediterranean sea in 2011 using a novel Universal Probe Library (UPL) RT-PCR assay*. Veterinary Microbiology, 165: 109-114.
- 2013. Carballo, M. , **Esperón, F.** , Sacristán, C. , González, M. , Vázquez, B. , Aguayo, S. and Torre, A. *Occurrence of tetracycline residues and antimicrobial resistance in gram negative bacteria isolates from cattle farms in Spain*. Advances in Bioscience and Biotechnology, 4: 295-303.
- 2012. Arbelo, M.; Bellière, E.N.; Sierra, E.; Sacchinni, S.; **Esperón, F.**; Andrada, M.; Rivero, M.; Diaz-Delgado, J.; Fernández, A. *Herpes virus infection associated with interstitial nephritis in a beaked whale (Mesoplodon densirostris)*. BMC Veterinary Research 13;8:243.
- 2012. **Esperón, F.**; García-Párraga, D.; Bellière, E.N.; Sánchez-Vizcaíno, J.M. *Molecular diagnosis of lobomycosis-like disease in a bottlenose dolphin in captivity*. Medical Mycology, 50(1):106-9.
- 2011. Bellière, E.N.; **Esperón, F.**; Sánchez-Vizcaíno, J.M. *Genetic comparison among dolphin morbillivirus in the 1990-1992 and 2006-2008 Mediterranean outbreaks*. Infection and Genetics Evolution, 11(8):1913-20.
- 2011. Bellière, E.N.; **Esperón, F.**; Fernández, A.; Arbelo, M.; Muñoz, M.J.; Sánchez-Vizcaíno J.M. *Phylogenetic analysis of a new Cetacean morbillivirus from a short-finned pilot whale stranded in the Canary Islands*. Research in Veterinary Science, 90: 324-328

Other relevant facts.

- Edwige Nina Bellière. “Advances on cetacean virology: molecular characterization of new sequences of Morbilliviruses and Herpesviruses”. PhD thesis. Directors: J.M. Sánchez-Vizcaíno and F. Esperón. October, 2012.
- Esperón F, Bellière E, Sánchez-Vizcaíno J (2010) Chapter 4: New perspectives on the Cetacean morbillivirus epidemiology and taxonomy. In: Pearce AG, Correa LM (eds) Dolphins: Anatomy, Behavior and Threats (Animal Science, Issues and Professions). Nova Science Publishers, Inc., p 107-128.



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Our Ref:

Your Ref:

To the Morris Animal Foundation,

28th November, 2014

Date:

RE: LETTER OF SUPPORT FOR THE PROJECT A FIRST PHYLOGENETIC AND SPATIAL ANALYSIS OF PARVOVIRUS STRAINS CIRCULATING IN THE SERENGETI MAASAI MARA ECOSYSTEM.

The Tanzanian Wildlife Research Institute (TAWIRI) is aware of the project entitled, "*A first phylogenetic and spatial analysis of parvovirus strains circulating in the Serengeti Maasai Mara ecosystem*".

The Serengeti Maasai Mara ecosystem is a protected area of international importance within which African carnivores play a pivotal role. As a consequence their conservation is of great value. However the influence of human settlements and the transmission of infectious diseases from domestic to wild species are principal threats for wild carnivore populations and the mitigation of these impacts will be key to their conservation.

Current mass dog vaccination programmes are being undertaken in this environment with parvovirus one of the vaccines used. However, there have been no studies in the Serengeti Maasai Mara ecosystem about the epidemiology of this pathogen, resulting in a knowledge gap regarding the strains of the virus that are circulating, the susceptibility of carnivore species to infection, and the broader impact that vaccination of domestic dogs against parvovirus has on wild carnivores.

Given these issues, TAWIRI supports this project's application to the Morris Animal Foundation Grants in 2014 to seek funding to support this important work.

Yours sincerely,

TANZANIA WILDLIFE RESEARCH INSTITUTE

Dr. Angela Mwakatobe
FOR: DIRECTOR GENERAL

TAWIRI is responsible for the co-ordination of all wildlife research in Tanzania

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20thth November, 2014

The Morris Animal Foundation,

Dear Sir/Madam,

Re: Letter of support for the project A first phylogenetic and spatial analysis of parvovirus strains circulating in the Serengeti Maasai Mara ecosystem

The Nelson Mandela African Institution of Science and Technology (NMAIST) is aware of the project entitled A first phylogenetic and spatial analysis of parvovirus strains circulating in the Serengeti Maasai Mara ecosystem.

The Serengeti Maasai Mara ecosystem is a protected area of international importance within which African carnivores play a pivotal role. As a consequence, their conservation is of great value. However the influence of human settlements and the transmission of infectious diseases from domestic to wild species are principal threats for wild carnivore populations and the mitigation of these impacts will be key to their conservation.

Current mass dog vaccination programmes are being undertaken in this environment with parvovirus one of the vaccines used. However, there have been no studies in the Serengeti Maasai Mara ecosystem about the epidemiology of this pathogen, resulting in a knowledge gap regarding the strains of the virus that are circulating, the susceptibility of carnivore species to infection, and the broader impact that vaccination of domestic dogs against parvovirus has on wild carnivores.

Given these issues, NMAIST *REQUESTS* to the Morris Animal Foundation Grants in 2014 to seek funding to support this important work. If the funding application is successful, and the study proceeds, we expect that NMAIST students will be able to collaborate on, and benefit from, the project.

Yours sincerely,

Prof. Joram Buza, Dean of the School of Life Sciences



18th November 2014

Project Title:

A first phylogenetic and spatial analysis of parvovirus strains circulating in the Serengeti Maasai Mara ecosystem

To Whom It May Concern:

This is a letter of support for the above project. Over the past 20 years research work that colleagues and I have conducted in the Serengeti ecosystem has resulted in a functional research network being established and many biological samples, currently stored in a frozen archive in Tanzania, being collected.

We are happy to offer this project the support of our Tanzanian infrastructure to collect new samples and to grant the PI access to our archive as we feel that the project's objectives will be of value to disease surveillance efforts in the ecosystem. Moreover the samples within our bank are there to be used and this project provides an excellent opportunity to answer some fundamental questions about the ecology of parvovirus. We therefore give our full support to the project and hope that the funding application is successful.

Yours truly,

Prof. Sarah Cleaveland

Nelson Mandela African Institution of Science & Technology
Arusha
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And

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