Adenoviruses in Côte d`Ivoire: investigation of diversity and interspecies transmission

von Maude Suzanne Pauly

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Adenoviruses in Côte d`Ivoire: investigation of diversity and interspecies transmission

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This thesis is dedicated to Mamama and Metto,

who will always walk beside me and will stay forewer in my heart.

Three first author publications based on data collected and analyses performed within the framework of the thesis, were published: "*High prevalence and diversity of species D AdV (HAdV D) in human populations of four Sub-Saharan countries.*" (Pauly *et al.* 2014), "*Adenovirus in Rural Côte D`Ivoire: High Diversity and Cross-Species Detection."*(Pauly *et al.* 2015) and *"Contact with non-human primates and risk factors for zoonotic disease emergence in the Taï region, Côte d`Ivoire."(Mossoun et al. 2015)* (shared first authorship). Several sections of the papers were slightly adopted or directly copied in this thesis.

A student in biotechnology of the University of Applied Sciences (Beuth Hochschule für Technik, Berlin), Nanina Buchwald, did some of the analyses (first AdV screening of the poultry and of the rodents) for her bachelor thesis and was supervised by Maude Pauly and Dr. Bernhard Ehlers.

Three PhD students, two from Côte d`Ivoire (Etile Anoh Augustin and Arsène Mossoun), and myself were engaged in the entire project, participated in the field missions and analyzed the collected data. The statistical analysis was done with the scientific support of Siv-Aina Leendertz and the data processing with the help of Arsène Mossoun.

1. Introduction

Introduction: English Version

In order to achieve the sixth Millennium Development Goal ("fighting infectious diseases and specifically controlling neglected tropical diseases by 2015") (MDG Africa Steering Group 2008), there is an urgent need for research in tropical Africa, where 53 % of infectious disease outbreaks occur (Chan *et al.* 2010). Until completion of knowledge on dynamics of infectious diseases (comprising the ecological, evolutionary, social, economic, and epidemiological mechanisms), reduction of morbidity and mortality caused by infectious diseases will unlikely be met. Special focus should be put on viral zoonoses, which ''are diseases or infections naturally transmitted between vertebrates and humans'' (World Health Organization). More than 25 % of the emerging infectious diseases (EID) are caused by viruses and the majority originated from animal hosts (Jones *et al.* 2008, Howard and Fletcher 2012). It has been shown that there is a significant disequilibrium concerning global disease surveillance (Jones *et al.* 2008): too few scientific resources are invested in countries with high biodiversity and low infrastructure, which represent hotspots for disease emergence (e.g. sub-saharan Africa). Thus, it is of paramount importance to develop the capacities of these countries to react quickly and effectively to emerging disease outbreaks as the consequences can have fatal effects on human and animal health, on economy, trade and tourism (Epstein and Price 2009).

In sub-Saharan Africa, frequent, long-lasting and intense interspecies contacts increase the risk for interspecies transmission of pathogens. Agriculture and breeding of domestic animals modified pathogen ecology by creating novel livestock-wildlife-human interactions and the perfect environment for the development and spread of pathogen vectors (Pearce-Duvet 2006). In several contexts, host switches could be associated with anthropogenic modifications of the environment or with bushmeat related activities (Wolfe *et al.* 2005, Engel *et al.* 2006). Host switches often lead to increased pathogenicity of the pathogen in the new unadapted host population (Parrish *et al.* 2008). Altered pathogenicity, infectivity, transmissibility and tropism can also be due to recombinant infectious agents emerging after frequent and intense exposure to diverse agents (Walsh *et al.* 2009, Dehghan *et al.* 2013). To mitigate disease threats to public health, livestock economies and wildlife conservation, extensive pathogen-specific risk analyses with a multidisciplinary approach have to be conducted in tropical Africa.

Against this background, a project on "Capacity Building for the Detection and Prevention of Neglected Zoonotic Infectious Diseases in Tropical Africa" was designed by an international and multidisciplinary team. The two main study regions, the Taï region in Côte d`Ivoire (CI) and the Salonga National Park in Democratic Republic of the Congo (DRC) are remote, rural regions, for which information on incidence and prevalence of infectious diseases is limited. As the local population consists of breeders, cultivators and hunters, contact to livestock and wildlife is frequent and intense. It is thus the perfect environment to investigate whether overlapping human and animal habitat can influence rates and patterns of pathogen transmission between humans, livestock and wildlife. Investigated pathogens in the project were retroviruses, cytomegaloviruses and adenoviruses (AdV). All of them are endemic in tropical Africa and can cause disease in (immunosuppressed) human and animal host.

The present study represents the part of the project devoted to the study of AdV. AdV have been detected in mammals, birds, fishes, amphibians and reptiles, worldwide. Most AdV infections are asymptomatic in human and animal hosts. However, AdV-induced symptoms include gastroenteritis, kerato-konjunctivitis and pneumonitis. Bacterial co-infection, young age and immunosuppression enhance the risk to develop severe symptoms. More recently, zoonotic transmissions have been repeatedly reported for different AdV, and AdV recombinants have been detected (Horwitz and Wold

2007, Harrach *et al.* 2008, Hoppe *et al.* 2015, Hoppe *et al.* 2015). Little is known about epidemiology, phylogeny and pathogenicity of AdV in humans and animals living in the Taï region in Côte d`Ivoire. As AdV prevalence and diversity have already been described in non-human primates of the region (Wevers *et al.* 2011, Hoppe *et al.* 2015), the present thesis focused mainly on humans and domestic animals (livestock and companion animals). It has been shown that livestock can play key roles as intermediate host or as "bridge" for the transmission of wildlife pathogens to humans, as they are more likely to come into contact with wildlife (Daszak *et al.* 2000, Pearce-Duvet 2006, Wood *et al.* 2012).

The general goal of this PhD thesis was to identify and estimate the risk for cross-species transmission of AdV between humans and domestic animals. The gathered knowledge may be beneficial in formulating prevention recommendations to reduce pathogen transmission in areas where humans, livestock and wildlife cohabit. Based on the general goal of the thesis, the particular aims of the present study were:

1)To determine the type and prevalence of AdV in feces of humans and domestic animals in rural Côte d`Ivoire

2) To perform phylogenetic and recombination analysis of the identified sequences to characterize the circulating AdV strains.

3) To statistically analyze behavioral and demographic data to identify risk factors for zoonotic disease transmission

4) To assess the occurrence of zoonotic transmission of AdV

5)To unravel factors that might facilitate AdV spread, and symptoms associated with adenoviral infection.

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List of abbreviations

AASV	Avian AdV Splenomegaly virus
AdV	Adenovirus or Adenoviruses
AIC	Akaike information criterion
AIDS	Acquired ImmunoDeficiency Syndrome
BEAST	Bayesian Evolutionary Analysis by Sampling Trees
BLAST	Basic Local Alignment Search Tool
bp	Base pair
CAR	Central African Republic
CAR	Coxsackie-AdV receptor
CAV	Chickens Anaemia Virus
CD	Cluster of Differentiation
CDS	Coding DNA sequence
CELO	Chickens Embryo Lethal Orphan Virus
CI	Côte d`Ivoire
CNER	Comité National d'Ethique et de la Recherche
cytb	Cytochrome b
DFG	Deutsche ForschungsGemeinschaft
DNA	DeoxyriboNucleic Acid
DPOL	DNA Polymerase
DRC	Democratic Republic of the Congo
E gene	early gene
EDS	Egg Drop Syndrome
EID	Emerging Infectious Disease
ELISA	Enzyme-Linked Immunosorbent Assay
F	Female
FAdV	Fowl adenovirus
GenDist	Genetic Distance
GLM	Generalized Linear Model
GMYC	Generalized Mixed Yule Coalescent
GTR	General Time Reversible nucleotide substitution model
HAdV	Human AdV
HEV	Hemorrhagic Enteritis Virus
HEX	HEXon gene
HHS	Hepatitis Hydropericardium Syndrome
HIV	Human Immunodeficiency Virus
HKY	Hasegawa-Kishino-Yano
HPS	Hydropericardium syndrome
HTLV	Human T-Lymphotropic Virus
IBDV	Infectious Bursal Disease Virus
IBH	Inclusion body hepatitis
ICH	Infectious Canine Hepatitis
ICTV	International Committee on Taxonomy of Viruses
ITB	Infectious TracheoBronchitis
ITR	Inverted Terminal Repeat
kbp	kilo base pair
L gene	late gene
LANADA	Laboratoire National d'Appui au Dévelopment Agricole
M	Male
MCMC	Markov chain Monte Carlo integration
MDG	Millennium Development Goal

minGD	minimum Genetic Distance
MLP	Major Late Promoter
MLTU	Major Late Transcription Unit
mRNA	Messenger RNA
MSD	marble spleen disease
MSDV	Marble spleen disease virus
N°	Number
NCBI	National Center for Biotechnology Information
neg	negative
NGO	Non-Governmental Organization
NGVEV	New-type Gosling Viral Enteritis Virus
NHP	Non-Human Primate
nt	nucleotide
ONUCI	United Nations Operation in Côte d`Ivoire
ORF	Open Reading Frame
PAdV	porcine AdV
PCR	Polymerase Chain Reaction
pos	positive
*R*0	Reproductive ratio
RDP	Recombination Detection Program
RKI	Robert Koch-Institute
RNA	Ribonucleic acid
S	simian
s and as primer	sense and antisense primer
SAdV	Simian adenovirus
SARS	Severe Acute Respiratory Syndrome
SFV	Simian Foamy Virus
SIV	Simian Immunodeficiency Virus
STLV	Simian T-Lymphotropic Virus
TAdV	Turkey AdV
Tm*	Annealing temperature
TP	Terminal Protein
UG	Uganda
WCF	Wild Chimpanzee Foundation
WHO	World Health Organization
YOPI	Young, Old, Pregnant, Immunosuppressed
95%CI	95 % confidence interval

2. Literature Review

2.1 Adenoviruses: General features

History. In 1953, Wallace Rowe, while investigating the "virus of the common cold" isolated a new virus in explants of human adenoid tissue, grown in cell culture (Rowe *et al.* 1953). Only a year later, Maurice Hilleman identified a new pathogen causing the Acute Respiratory Disease of military recruits, and closely related to the agent discovered by Rowe before (Hilleman and Werner 1954, Ginsberg *et al.* 1955). Only in 1956, this new virus got the to date accepted name "Adenovirus"(AdV), derived from the Greek word for "gland", the tissue in which the virus was first isolated (Enders *et al.* 1956). Only later, Adenoviruses (AdV) were isolated from tissues of different animal species, including e.g. cattle, horses, turkeys and mice (Klein *et al.* 1959, Hartley and Rowe 1960, Ardans *et al.* 1973, Carlson *et al.* 1974).

Taxonomy and Classification. These non-enveloped, icosahedral, linear and double-stranded DNA-viruses have been detected in members of every vertebrate class (Harrach *et al.* 2011). The AdV taxonomy and classification into genera is still based on the concept that AdV are host-specific and have coevolved with their vertebrate host: *Mastadenovirus* coevolved with mammals (*Mammalia*), *Aviadenovirus* with birds (*Aves*), *Siadenovirus* with amphibians (*Amphibia*), *Ichtadenovirus* with fish (*Ichthyes*) and *Atadenovirus* with scaled reptiles (*Squamata*) *(Table 1)(Benkö and Harrach 2003, Davison et al. 2003, Kovacs and Benkö 2011)*. In fact all human and most mammalian AdV belong to the genus *Mastadenovirus* and most avian AdV to the genus *Aviadenovirus.* However, the genera, *Atadenovirus* and *Siadenovirus* have a broader host range*. Atadenoviruses* have been isolated in reptiles, various ruminants, birds and a marsupial (Benkö and Harrach 1998, Benkö *et al.* 2002). It has been hypothesized that atadenoviruses might have adapted to avian hosts later (Farkas *et al.* 2002). Thus far, no animal class could be assigned to *Siadenovirus* and only 5 recognized siadenoviruses of avian (isolated in raptor species, in a great tit, in a south polar skua, in turkeys) and one of frog origin have been detected (Davison *et al.* 2000, Kovács and Benkő 2009, Wellehan *et al.* 2009, Kovacs *et al.* 2011, Park *et al.* 2012). More siadenoviruses have been detected in psittacine birds, in tortoises and in a budgerigar (Katoh *et al.* 2009, Rivera *et al.* 2009, Wellehan *et al.* 2009). The fifth genus I*chtadenovirus* comprises the so far unique fish AdV, which was isolated from a white sturgeon (*Acipenser transmontanus*) (Kovács *et al.* 2003). Recently a putative new genus, testinoid AdV, was proposed based on phylogenetic analyses of AdV isolates detected in different tortoise species (Doszpoly *et al.* 2013).

The prefix "Mast" of *Mastadenovirus* was derived from the Greek "*mastos*", the word for "breast"; the prefix "Avi" of *Aviadenovirus* was derived from the Greek "*avis*", the word for "bird" and the prefix "Icht" of *Ichtadenovirus* from the Greek "*ichthys*", the word for "fish". The designation of the other two genera was chosen in recognition of their genus-specific genome structure or of their genus-specific enzyme content: atadenoviruses have a high A+T content and siadenoviruses have a gene encoding a putative sialidase homolog.

Genus	Mastadenovirus	Aviadenovirus	Siadenovirus	Atadenovirus	Ichtadenovirus
Designation	Greek for "breast"	Greek for "bird"	putative sialidase homolog	high AT content	Greek for "fish"
Host species	mammal	bird	amphibian, bird	bird, reptile, ruminant, marsupial	fish
N° of recognized species	25	8	3	5	1
N° of recognized types	>89	>14	>3	>9	1
Genome length	30-39 kbp	44-46 kbp	approx. 26 kbp	30-33 kbp	approx. 48 kbp
G+C content	44-64 %	54- 67 %	35-39%	34-43 %	approx. 42%
Specificities	unique proteins, among others proteins V and IX	2 fibers per vertex; longest genome; no E3 region	protein related to sialidases; low G+C content	high A+T content; unique protein p32K, LH1-3 and RH	fiber gene homolog at the left end of the genome

Table 1.Comparison of the AdV genera; data derived from the 9th report of ICTV on virus taxonomy (Harrach et al. 2011)

Each genus is further divided into several AdV species, comprising different AdV types. Historically, species determination was based on serological and biological properties (host species, oncogenic, haemagglutinating and morphological properties). The different species show an amino acid sequence difference of hexon and DNA polymerase gene of at least 5-10 %. Other criteria proposed by the ICTV, are for example DNA hybridization, restriction fragment length polymorphism, nucleotide composition and oncogenicity in rodents *(Table 2)* (Harrach *et al.* 2011). For instance, the human AdV types are grouped into seven species*,*human AdV A-G (HAdV A-G) and the genus *Aviadenovirus* into 5 species: falcon AdV A, Goose AdV A, Turkey AdV B and Fowl AdV A-E (FAdV A-E)(Harrach *et al.* 2011). Pigeon AdV A (PiAdV-1) and Duck AdV B (DAdV-2) are candidate species in the genus *Aviadenovirus (Marek et al. 2014)*.

In the 9th report of ICTV on virus taxonomy, the criterion for serotype differentiation was specified. The differentiation is mainly based on neutralization assays: "*A serotype is defined as either exhibiting no cross-reaction with others or showing homologous/heterologous titer ratios of 8 or 16*". Moreover serotypes presenting substantial biophysical, biochemical, or phylogenetic differences are considered to be distinct (Harrach *et al.* 2011). Hower other criteria for (geno-) type demarcation have been proposed. As the antigens at the surface of the virion are mainly type-specific, characterization of these antigens alone can be sufficient for type identification. Examples are the hexon and fiber proteins of the viral capsid. Both are involved in neutralization inhibition. Additionally, fibers are involved in hemagglutination inhibition. While the genus-specific antigen is located on the basal surface of the hexon, the serotype-specific antigens are located mainly on the tower region of the hexon. Phylogenetic analyses of complete sequences of the capsid proteins, hexon, fiber and penton base, have been shown to be good predictors for new types and for detection of recombination events (de Jong *et al.* 2008, Maluquer de Motes *et al.* 2011, Singh *et al.* 2012). An important criterion permitting the AdV classification and recognition is molecular divergence within the hexon and DNA polymerase gene (Wellehan *et al.* 2004, Ebner *et al.* 2005, Madisch *et al.* 2005, Wevers *et al.* 2011). Criteria for typing of the human AdV based on genomics and complete genome data have been proposed by the Human AdV Working Group (http://hadvwg.gmu.edu/).

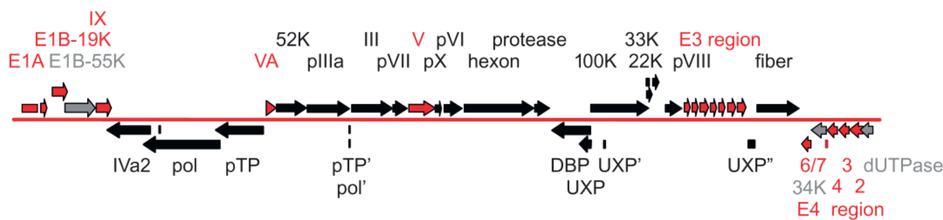
Genus	Species	Type example	Pathogenicity of AdV species
Mastadenovirus	Human Mastadenovirus A	HAdV-12	Gastro-intestinal diseases in children?
	Human Mastadenovirus B	HAdV-3	Respiratory disease, hemorrhagic cystitis, Neurological symptoms; conjuntivitis
	Human Mastadenovirus C	HAdV-1	Endemic infection, respiratory symptoms
	Human Mastadenovirus D	HAdV-8	Epidemic keratoconjunctivitis
	Human Mastadenovirus E	HAdV-4	Respiratory disease, conjunctivitis
	Human Mastadenovirus F	HAdV-40	Infantile diarrhea
	Human Mastadenovirus G	HAdV-52	Diarrhea?
	Bat Mastadenovirus A	Bat AdV-3	?
	Bat Mastadenovirus B	Bat AdV2	?
	Bovine Mastadenovirus A	Bovine AdV-1	
	Bovine Mastadenovirus B	Bovine AdV-3	Pneumo-enteritis, encephalitis, keratoconjunctivitis
	Bovine Mastadenovirus C	Bovine AdV-10	
	Canine Mastadenovirus A	Canine AdV-1,-2	Infectious canine hepatitis, kennel cough
	Equine Mastadenovirus A	Equine AdV-1	Pneumonia in immunocompromised animals
	Equine Mastadenovirus B	Equine AdV-2	Gastrointestinal infections
	Murine Mastadenovirus A	Murine AdV-1	?
	Murine Mastadenovirus B	Murine AdV-2	?
	Murine Mastadenovirus C	Murine AdV-3	?
	Ovine Mastadenovirus A	Ovine AdV-2	Enzootic bronchopneumonia, calf pneumo-enteritis
	Ovine Mastadenovirus B	Ovine AdV-1	
	Porcine Mastadenovirus A	Porcine AdV1	Mild upper respiratory disease
	Porcine Mastadenovirus B	Porcine ADV-4	Neurological disease
	Porcine Mastadenovirus C	Porcine AdV-5	Mild upper respiratory disease
	Tree shrew Mastadenovirus A	Three Shrew AdV-1	?
	Simian Mastadenovirus A	Simian AdV-3	Enteritis
Aviadenovirus	Fowl Aviadenovirus A	FAdV-1	Gizzard erosions
	Fowl Aviadenovirus B	FAdV-5	?
	Fowl Aviadenovirus C	FAdV-4	Inclusion body hepatitis, Hydropericardium syndrome
	Fowl Aviadenovirus D	FAdV-3	Inclusion body hepatitis
	Fowl Aviadenovirus E	FAdV-6	Inclusion body hepatitis, Hydropericardium syndrome
	Falcon Aviadenovirus A	Falcon AdV-1	?
	Goose Aviadenovirus A	Goose AdV-1	?
	Turkey Aviadenovirus B	TAdV-1	?

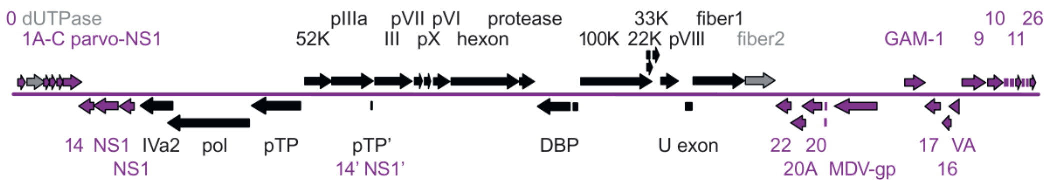
Table 2.Taxonomy of AdV. Genera and species currently recognized by the ICTV (Harrach et al. 2011)

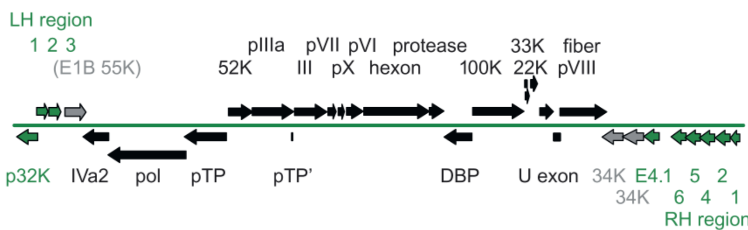
Siadenovirus	Frog Siadenovirus A	Frog AdV-1	?
	Great tit Siadenovirus A	Great tit AdV-1	?
	Raptor Siadenovirus A	Raptor AdV-1	?
	Skua Siadenovirus A	Skua AdV-1	?
	Turkey Siadenovirus A	TAdV-3	Haemorrhagic enteritis, Avian AdV Splenomegaly, Marble spleen disease
Atadenovirus	Bovine Atadenovirus D	Bovine AdV-4	Enteritis
	Duck Atadenovirus A	Duck AdV-1	Egg Drop Syndrome
	Ovine Atadenovirus D	Ovine AdV-7	Pneumonia
	Possum Atadenovirus A	Porcine AdV-1	?
	Snake Atadenovirus A	Snake AdV-1	?
Ichtadenovirus	Sturgeon Ichtadenovirus A	Sturgeon AdV-1	?

Table 2 continued

Genome organization. AdV have an icosahedral capsid of 70-90nm in diameter and an inner nucleoprotein core. The linear AdV genome is on average 36 kbp long with an average G+C content of 51 % and contains an inverted terminal repeat (ITR) of 36-200 bp at each terminus (Harrach *et al.* 2011). A virus-coded protein, the terminal protein (TP) is attached to the ITR at the 5`-end and facilitates the binding of the polymerase to the origin. The average genome length of *Mastadenovirus* is 34 kb, of *Aviadenovirus* 45 kb, of *Siadenovirus* 26 kb, of *Atadenovirus* 32 kb and of *Ichtadenovirus* 48kb. While the genetic organization of the central part of the genome of the *Adenoviridae* is well-conserved within the genera, the genome ends have differing lengths and gene layouts (Davison *et al.* 2003). The central part contains the genus-common genes, which encode structural and replication proteins and whose transcription is driven by the major late promoter (MLP). The flanking region contains the genus-specific genes, which encode regulatory proteins. The only exception for this basic genome plan is the genus *Ichtadenovirus* (Harrach *et al.* 2011). Splicing has been extensively investigated for AdV and plays an important role for mRNA expression. In spite of example, most late genes in the conserved region are expressed by splicing from the rightward-oriented MLP located in the polymerase gene (*Figure 1)*.







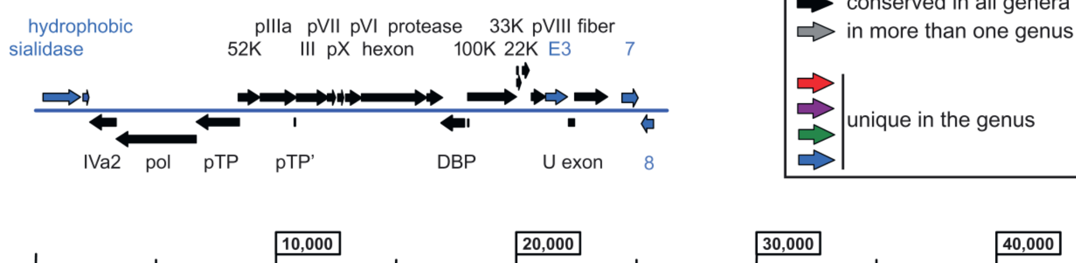


Figure 1. Schematic illustration of the various genome organizations found in members of four adenovirus genera.

Black arrows depict the genes conserved in every genus, grey arrows show genes present in more than one genus, and coloured arrows show genus-specific genes {From Ninth Report of ICTV (Harrach et al. 2011)}.

Structure and Function. The virus capsid consists of the hexon and the penton proteins with the protruding fiber protein. In total the capsid is built of 252 capsomers (Russell 2009). The facets of the capsid are composed by trimers of the hexon protein and other minor proteinic components.The hexon protein is the major capsid protein and comprises 240 nonvertex capsomers(Harrach *et al.* 2008). The penton protein, which is located at the vertices of the capsid, comprises 12 vertex capsomers (Russell 2009). The proximal end of the fiber is the pentameric penton base and the distal end forms the globular “knob” domain (Zhang and Bergelson 2005). In contrast to most members of the genus *Mastadenovirus*, some species of the genus *Aviadenovirus* present two fiber proteins per vertex penton (Gelderblom and Maichle-Lauppe 1982). Two fibers have also been described for members of the species HAdV F and species HAdV G (Jones *et al.* 2007).

The below discussed viral life cycle is based on studies carried out on HAdV-2 and -5, but applies to most AdV with only subtle variations. The fiber and penton proteins are important for virus entry: for attachment on cellular receptors and internalization in an endocytic vesicle. For a successful virus entry, the fiber knob must attach on an adapted receptor, and the penton base must interact with cell-surface integrins (Harrach *et al.* 2008, Russell 2009). The fiber of the HAdV species A, C, D, E and F and of some animal AdV attaches to the coxsackievirus B and AdV receptor (CAR), which is a cell-surface protein of the immunoglobulin superfamily (Zhang *et al.* 2005). Besides, CAR functions as cell-to-cell adhesion molecule and can be found between adjacent cells of the epithelium. It is only after the interruption of this connection that virus can increasingly infect the host (Zhang *et al.* 2005). Other cell surface molecules have been discussed to play a role as receptors for AdV under particular circumstances, e.g. CD 46, CD 80 and CD86 receptor for HAdV B and sialic acid for some HAdV D (Zhang *et al.* 2005). After the lysis of the endosomal membrane, the residual particles enter the cytoplasm and are transported along microtubules toward the nucleus. The remaining virus particles serve as template for virus transcription, which takes place in the nucleus. The replication mechanism of AdV is completely distinct from that of its host organism and only few host proteins are involved, e.g. RNA polymerase II. All components of the particle are encoded by the virus and both genome strands are used to encode proteins from genes. Basically, the adenoviral genome is constituted of the terminal ITRs, the early transcription units (E1A, E1B, E2, E3, E4) and the major late transcription unit (MLTU)(MLP, L1-L5). The different regions of the genome are only gradually activated by viral and host proteins and then expressed. The early (E) genes are expressed before, and late (L) genes after replication of the viral chromosome.

In the following the gene activation cascade of AdV will be described. E1 gene products control the viral gene transcription; inhibit cellular proteins and cellular transformation. Only after transcription of the E1A gene, other early gene promoters are activated and the remaining early transcriptions units (E1B, E2, E3, and E4) are expressed. They encode the proteins required for DNA replication (e.g. DNA polymerase) and host environment modulation (activation of cell cycle, inhibition of cell cycle control, inhibition of programmed cell death, bypassing of the host immune response etc.). Moreover the early genes are responsible for a consequent transcription and translation of the late genes (MLTU). The MLTU comprises genes that encode mainly genus-specific structural proteins, and other proteins, involved in virion assembly. All the resulting mRNAs are derived from a single pre-mRNA (a bi-or tripartite leader) by alternative splicing and polyadenylation. Virion assembly takes place in the nucleus and virions are released by cell lysis (Harrach *et al.* 2008, Russell 2009).

AdV as vector. Early on, advantages of AdV for vector development were recognized; among others, their ability to infect a wide variety of cell types, including dividing as well as nondividing cells and the simple manipulation of their genome. At first they were used in tumor-therapy: for treatment of genetic diseases by the delivery of foreign genome into mammalian cells. However the rapid induction of innate and adoptive cell-mediated and humoral immune response against antigens of AdV, prohibited the long-term gene expression of such AdV based vectors (Russell 2009). Yet this property of AdV made them highly attractive as vaccine carriers (Jooss and Chirmule 2003, Ndi *et al.* 2013). Several vaccines, based on human and animal adenoviral vectors, have been, and are being developed for vaccination against various human (e.g. Ebola, Malaria, HIV)(Bridgeman *et al.* 2009, de Wit *et al.* 2011, Bruder *et al.* 2012) and animal infections (e.g. rabies, bovine tuberculosis) (Ndi *et al.* 2013). A major disadvantage of many AdV based vaccine carriers is that many humans and animals

possess pre-existing antibodies against the used vector after natural AdV infection. To circumvent such pre-existing immunity, vectors based on rare animal (mainly non-human primate) AdV types have been tested (Lasaro and Ertl 2009). It has been demonstrated that there is no immunological cross-reactivity among human and animal AdV (Sharma *et al.* 2010). Another advantage of animal AdV based vectors with respect to the safety of vector systems, is that, although animal AdV seem to be able to infect non-animal cells, the productive replication cycle is rarely completed (Kumin *et al.* 2002). Examples for vectors based on animal AdV are the recombinant ovine, porcine and canine AdV, which have been tested as alternatives for gene transfer (Hofmann *et al.* 1999, Bangari and Mittal 2004, Bru *et al.* 2010, Patel *et al.* 2010). Similarly human AdV have been tested as adenoviral vectors for animals (Prevec *et al.* 1989).

Biological Properties. The pathology of AdV is due to their potential to stimulate cellular proliferation as a mechanism to facilitate virus replication and also to induce cell lysis by apoptosis. However, apoptosis is blocked during early infection by several viral proteins. The adaptive immune response is impaired as adenoviral proteins prevent the export of the mature major histocompatibility complex I antigens to the cell membrane. Other effects, such as toxic effects have been discussed. Large amounts of virions, concentrated within the nucleus, lead to early rounding of cells and aggregation or lysis of chromatin. This cytopathology can be visualized with electron microscopy and the so-called eosinophilic or basophilic intranuclear inclusion bodies are characteristic for infected cells(Harrach *et al.* 2008).

AdV are opportunistic or facultative pathogens. Although the majority of the AdV infections occurs during early life, is self-limiting and is not associated with disease, life-threatening outbreaks have been reported (Murphy *et al.* 1999, Horwitz *et al.* 2007). Hosts at risk for development of severe adenoviral diseases are immunocompromised (AIDS, organ transplantation) or present a concurrent infection with other agents; hence mainly the YOPI (young, old, pregnant, immunosuppressed) population. In the animal host, management problems, such as crowding or insufficient hygiene measures can lead to fatal adenoviral diseases (Harrach *et al.* 2008). Crowding has also been reported as predisposing factor for humans, e.g. infected military recruits in the United States, who developed severe respiratory symptoms (Kajon *et al.* 2007). Fact is that in such crowded population aerosol transmission of AdV is facilitated. AdV are mainly transmitted horizontally via direct or indirect oral-faecal route. Additional transmission routes via throat, eye, and urine occur. Vertical transmission of AdV has been reported in various hosts {e.g. transplacental transmission of bovine AdV (Bartha and Mate 1983)}. Especially for FAdV vertical transmission plays a significant role for virus spread (McFerran and Smyth 2000, Grgic *et al.* 2006). AdV can be excreted for several weeks after the primary (symptomatic) infection and can stay latent in lymphatic tissues of tonsils, adenoids and intestinal tract (McFerran *et al.* 2000, Garnett *et al.* 2002, Garnett *et al.* 2009, Roy *et al.* 2011). Shedding is even prolonged in immunocompromised hosts (Kojaoghlanian *et al.* 2003). The estimated incubation period is 1-7 days and is probably dose dependent. For FAdV experimental incubation periods of 2-18 days have been reported (Ganesh and Raghavan 2000). The neutralizing antibodies produced after an infection with a certain AdV type are type-specific and do not cross-react with other serotypes. In contrast, the induced T-cell response seems to be less specific (Sharma *et al.* 2010). Besides, AdV induce potent inflammatory responses (Lasaro *et al.* 2009). AdV are very stable in the environment and can resist to temperature and pH moderations, and to lipid solvents and disinfectants. Hence waterborne infections are very common. AdV are the perfect candidates for

tracing of fecal environmental contamination (Sibley *et al.* 2011), as phylogenetic analyses of the detected AdV types offer the possibility to ascertain the primary host and to elucidate the pollution source (Maluquer de Motes *et al.* 2004, Hundesa *et al.* 2006, Wolf *et al.* 2010). Across the world AdV have been detected in waste- and river-water and in manure (Fong and Lipp 2005, Amdiouni *et al.* 2012, Sibanda and Okoh 2012) and swimming-pools are considered to be a widespread source of symptomatic AdV infections. Consequently, AdV were, besides caliciviruses, coxsackieviruses, and echoviruses, included in the “Candidate Contaminant List” as part of the Safe Drinking Water Act by the U.S. Environmental Protection Agency (http://water.epa.gov/scitech/drinkingwater/dws/ccl/ccl3.cfm).

Treatment and prevention. No specific treatment is available against AdV infection and thus prevention is of paramount importance to avoid AdV transmission and disease. A measure to prevent the AdV transmission is the application of strict hygienic precautions and a measure to prevent severe adenoviral diseases is the implementation of an immune-prophylactic program. Vaccines against AdV have been implemented in humans and animals. In the USA for example an orally administrable, live, enteric-coated vaccine against HAdV 4,7,21 is used in military units to prevent AdV-induced respiratory outbreak (Harrach *et al.* 2008, Hoke and Snyder 2013). In the poultry industry, vaccination programs for parental flocks of meat and laying lines of hens can comprise vaccination against AdV (Toro *et al.* 2002, Koncicki 2006); worldwide, dogs are vaccinated with a live or killed canine AdV component (Murphy *et al.* 1999, Schultz 2006).

2.2 Mastadenovirus

2.2.1 Genus specific features

Thus far, AdV from genus Mastadenovirus have only been isolated in mammals. By serology, they are distinguishable from other genera on the basis of different genus-specific complement-fixing antigens. Other differentiation criteria are characteristic genome organization and phylogenetic distance. The genome length of mastadenoviruses is between 30, 536 bp and 37, 860 bp and the G+C content between 43.6 and 63.9 %. The ITRs of mastadenoviruses are longer and more complex than those of other genera. Genus-specific proteins are the proteins V, IX and most of those coded by the early genes, precisely the E1A, E1B, E3 and E4 regions.

Each genus is further divided into several AdV species comprising different AdV types *(Table 1 and Table 2)*. For instance, the human AdV types are grouped into seven species: human AdV A-G (HAdV A-G) (Harrach *et al.* 2011).

2.2.2 Epidemiology in humans

AdV infections occur in humans around the world and nearly all adults have serologic evidence of past infections with one or more AdV. Although many AdV infections in humans are subclinical and

self-limiting, some human AdV have been associated with disease (Horwitz *et al.* 2007, Eckardt and Baumgart 2011). Particularly in immunocompromised hosts, such as bone marrow and solid organ transplant recipients and patients with human immunodeficiency virus (HIV), infection with human AdV (HAdV) or reactivation of persistent HAdV can lead to severe systemic diseases (Kojaoghlanian *et al.* 2003, Kampmann *et al.* 2005, Echavarria 2008).

For some HAdV the pathogenic role remains undetermined, for others a clear association with a certain disease could be made. The pathogenic role of **HAdV A** is questionable as it has been detected in asymptomatic patients as well as in patients with gastroenteritis (Harrach *et al.* 2008). **HAdV B1 and 2** however, are the causative agents of respiratory outbreaks in military recruits in the USA. Moreover, persistent interstitial infections in the kidney and hemorrhagic cystitis have been reported for HAdV B2 (Harrach *et al.* 2008). **HAdV C** is a major cause for respiratory infections (Walsh *et al.* 2011) and is the major HAdV species involed in chronic respiratory disease pathogenesis of children (Wurzel *et al.* 2014). Most HAdV types belong to species D and the majority of these **HAdV D** types have been detected in HIV-positive patients (Robinson *et al.* 2011, Matsushima *et al.* 2013, Robinson *et al.* 2013). Types D8, D19, D37 and D54 are known to cause epidemic keratoconjunctivitis (Takeuchi *et al.* 1999, Ariga *et al.* 2005, Ishiko *et al.* 2008, Kaneko *et al.* 2009, Kaneko *et al.* 2011). The virulent type D53, a recombinant between D8, D22, D37 and at least one unknown HAdV D type, showed a modified tropism and induced inflammation of the cornea (Walsh *et al.* 2009). Another recombinant type, D56, was involved in fatal pneumonia in a neonate and keratoconjunctivitis in three adults (Robinson *et al.* 2011).Type D36 has been associated with obesity in animals and humans (Arnold *et al.* 2010) and types D65 and D67 were detected in the stool of children with gastroenteritis (Matsushima *et al.* 2012, Matsushima *et al.* 2013). The pathogenicity of the HAdV D types frequently shed by patients with AIDS remains controversial (Hierholzer 1992, Khoo *et al.* 1995, Echavarria 2008, Curlin *et al.* 2010). Prolonged fecal and urinary shedding of different HAdV D types and of recombinants has been observed (Fox *et al.* 1977, Curlin *et al.* 2010). Besides HAdV B and C, **HAdV E** have been associated with acute respiratory diseases and pneumonia (Guo *et al.* 2012, Zou *et al.* 2012). **HAdV F** are, after norovirus, astrovirus and rotavirus, the most common viral pathogen involved in acute gastroenteritis in children (Djeneba *et al.* 2007, Filho *et al.* 2007, Al-Thani *et al.* 2013). The members of the more recently recognized species, **HAdV G**, were first isolated in patients with diarrhea and they may be part of the agents, causing gastroenteritis of unknown origin (Jones *et al.* 2007). Furthermore oncogenic potential has been reported for several AdV types. The oncogenicity might depend on factors such as virus dose, host age at infection and host immune status (Harrach *et al.* 2008). Especially in brain tumors, AdV from species B, D and C could be identified and the viruses might contribute to the tumor pathogenesis (Kosulin *et al.* 2007). Moreover, AdV from species C were frequently detected in lymphocytes infiltrating human sarcomas(Kosulin *et al.* 2013).

2.2.3 Epidemiology in mammals

Similarly to infected humans, respiratory and gastrointestinal symptoms have been described in infected equines, porcine, carnivores and ruminants (Pommer and Schamber 1991, Cutlip *et al.* 1996,

Smyth *et al.* 1996, Lehmkuhl and Cutlip 1999, DeBey *et al.* 2001, Lehmkuhl *et al.* 2001, Olson *et al.* 2004, Almes *et al.* 2010, Banyai *et al.* 2010, Cavanagh *et al.* 2012).

Most of the adenoviral diseases are not pathognomonic and thus AdV detection is necessary to confirm AdV as causative agent. *Mastadenovirus* shedding alone is only a hint and not an evidence for an infection with a pathogenic AdV strain, as AdV shedding has been frequently observed in subclinical mammals. The ingestion of fecally contaminated water or food is considered to be a major route of infection. Factors that enhance the risk for severe adenoviral diseases are management problems (mixing of animals from different origins), crowding, immunosuppression and concurrent bacterial infections.

The pathogenic role of many AdV still questionable and needs further investigation.

Ovine AdV and **bovine** AdV 2 isolates have been obtained from lambs with respiratory and gastrointestinal symptoms (Lehmkuhl and Cutlip 1984, Pommer *et al.* 1991, Lehmkuhl *et al.* 1993).

Similarly, **caprine** AdV have been isolated from goats with respiratory disease and enteritis(Olson *et al.* 2004), but also encephalitis (Lehmkuhl *et al.* 1999). In several African countries (Nigeria and Senegal) AdV was isolated from goats affected with peste des petits ruminants. However, the role of AdV in the epizootiology of peste des petits ruminants remains unresolved (Gibbs *et al.* 1977, Nguyen Ba *et al.* 1988, Durojaiye *et al.* 1991).

In cattle, **bovine** AdV could be associated with calf pneumo-enteritis, encephalitis, keratoconjunctivitis and acute febrile disease in cattle (Lehmkuhl *et al.* 1975, Smyth *et al.* 1996, Pardon *et al.* 2011).

In horses, the **equine** AdV-1 is predominantly associated with upper respiratory tract infections (Cavanagh *et al.* 2012). Particularly, in horses of the Arabian horse lineage with a primary severe combined immunodeficiency disease, severe AdV-induced diseases have been reported. As the affected animals are incapable to develop an accurate immune response, foals usually die of pneumonia due to equine AdV (McGuire *et al.* 1974). EAdV-2 appears to be associated with gastrointestinal infections in horses(Cavanagh *et al.* 2012).

Porcine AdV (mainly porcine AdV-3 and -4) are pathological agents involved in pneumonia, pneumo-enteritis or encephalitis (Edington *et al.* 1972, Ducatelle *et al.* 1982, Elazhary *et al.* 1985).

Canine AdV-1 and AdV-2 infections are epizootic in domestic and wild carnivores (foxes, bears, wolves, coyotes, and skunks). Canine AdV-1 is the causative agent of infectious canine hepatitis (ICH), and canine AdV-2 is one of the viral agents implicated in the aetiopathogenesis of infectious tracheobronchitis (ITB or kennel cough)(Murphy *et al.* 1999, Almes *et al.* 2010). Canines infected with canine AdV-1 can present mild symptoms (e.g. fever, depression and loss of appetite), but in severe cases, ICH is characterized by acute necrohaemorragic hepatitis. In the post-acute stage of the infection, circulating immune complex deposition may cause corneal oedema (blue eye), uveitis and interstitial nephritis. The principal symptoms of kennel cough (ITB), which can be exacerbated by bacterial co-infection, are cough, nasal discharge and fever (Murphy *et al.* 1999, Almes *et al.* 2010).The high seroprevalence of these viruses in several countries underlines the importance of these pathogenic, highly contagious agents for animal health (Levy *et al.* 2008, Mochizuki *et al.* 2008,

Philippa *et al.* 2008, Akerstedt *et al.* 2010, Qin *et al.* 2010, Balboni *et al.* 2013, Bulut *et al.* 2013). Since no specific therapy against symptomatic canine AdV infection is available, a vaccine, based on a modified live canine AdV-2 and cross-protecting against canine AdV-1, is the only possibility to reduce the risk of disease.

Feline AdV have been seldomly investigated. In one cat, suffering from transient hepatic failure, AdV could be repeatedly isolated from pharyngeal and rectal swabs and a disseminated AdV infection was reported for a cat with petechiae (Kennedy and Mullaney 1993, Lakatos *et al.* 1999, Lakatos *et al.* 2000).

Although **simian** AdV shedding has been described for asymptomatic monkeys and apes (Roy *et al.* 2009, Roy *et al.* 2012), it was also associated with severe disease, particularly in SIV (simian immunodeficiency virus) infected individuals. For instance, in SIV infected monkeys, AdV caused necrotizing hepatitis, pancreatitis and segmental enteritis (Baskin *et al.* 1988, Zoller *et al.* 2008). SAdV have been isolated from monkeys with diarrhea (Wang *et al.* 2007, Banyai *et al.* 2010), pancreatitis(Chandler and McClure 1982), and (fulminant) respiratory disease (Boyce *et al.* 1978, Chen *et al.* 2011, Chiu *et al.* 2013). The sole cotton-top tamarin (*Saguinus oedipus*) AdV was isolated in an individual with diarrhea (Hall *et al.* 2012).

Animals infected with HAdV-D36 showed increased adipose tissue and low levels of serum cholesterol and triglycerides (Dhurandhar *et al.* 2000, Dhurandhar *et al.* 2002, Arnold *et al.* 2010). In 1962, the tumorigenic effect of HAdV 12 in newborn hamsters was first shown. Since then HAdV infection, in particular HAdV A and B were shown to induce undifferentiated sarcomas, adenocarcinomas and neuroectodermal tumors in different rodent species (Graham *et al.* 1984, Thomas *et al.* 2006). In Lagomorpha, considerable morphological alterations and enhanced cell growth were observed after infection with HAdV(Wimmer *et al.* 2010).

Few data are available on the current prevalence of AdV in animals *(Table 3)*, but one can assume that AdV infections occur globally, as AdV infections have been detected in a high diversity of animal species, also from remote or isolated areas (e.g. Galapagos isolands) and from various ecosystems (e.g. Antarctica, the tropical rain forest) (Levy *et al.* 2008, Lee *et al.* 2014)

Animal species	Sample origin	AdV species	Detection method	Prevalence	Reference
arctic foxes	Norway	canine adV	serology	38 %	(Akerstedt *et al.* 2010)
bats	Kenya	bat AdV	PCR	2 %	(Conrardy *et al.* 2014)
	China	bat AdV	PCR	8 %	(Li *et al.* 2010)
	Hungary	bat AdV	PCR	5 %	(Janoska *et al.* 2011)
bonobos	different countries	different AdV	PCR	46 %	(Roy *et al.* 2009)
camel	Sudan	bovine AdV	serology	90 %	(Intisar *et al.* 2010)
cats	Czech Republiy	feline AdV	serology	35 %	(Lakatos *et al.* 1999)
cattle	USA	bovine AdV	PCR	13 %	(Sibley *et al.* 2011)
	USA	bovine AdV	serology	82 %	(Lehmkuhl and Hobbs 2008)
	Nigeria	different AdV	serology	4 %	(Obi and Taylor 1984)
	Zambia	bovine AdV	serology	87 %	(Ghirotti *et al.* 1991)
	Zaire (DRC)	bovine AdV	serology	44 %	(Jetteur *et al.* 1988)
	Spain	bovine AdV	PCR	75 %	(Maluquer de Motes *et al.* 2004)
	Irland	bovine AdV	serology	55 %	(Adair *et al.* 1996)
chimpanzee	different countries	different AdV	PCR	63 %	(Roy *et al.* 2009)
dogs	South Africa	canine AdV	serology	50 %	(Wright *et al.* 2013)
	Turkey	canine AdV	serology	28-100 %	(Gür and Acar 2009)
	Galapagos	canine AdV	serology	67 %	(Levy *et al.* 2008)
fox	Italia	canine AdV	PCR	9 %	(Balboni *et al.* 2013)
goat	Nigeria	different AdV	serology	18 %	(Obi *et al.* 1984)
	USA	caprine AdV	serology	60 %	(Lehmkuhl *et al.* 1999)
gorillas	different countries	different AdV	PCR	40 %	(Roy *et al.* 2009)
horses	Nigeria	different AdV	serology	5 %	(Obi *et al.* 1984)
mink	France	canine AdV	serology	2-10 %	(Philippa *et al.* 2008)
monkey	China	simian AdV	PCR	46 %	(Banyai *et al.* 2010)
macaque	China	simian and human AdV	PCR	46 %	(Wang *et al.* 2007)
non-human primates	Africa	simian and human AdV	PCR	59 %	(Wevers *et al.* 2011)
panda	China	canine AdV	serology	9 %	(Qin *et al.* 2010)
pigs	Spain	porcine AdV	PCR	70 %	(Maluquer de Motes *et al.* 2004)
	Quebec	porcine AdV	serology	15 %	(Dea and El Azhary 1984)
red fox	Norway	canine AdV	serology	57 %	(Akerstedt *et al.* 2010)

Table 3.Prevalence of AdV positivity in animals around the world

sheep	Nigeria	different AdV	serology	18 %	(Obi & Taylor, 1984)
	Australia	AdV	serology	71 %	(Peet et al., 1990)
	USA	caprine AdV	serology	80 %	(Lehmkuhl & Cutlip, 1999)
	Irland	bovine AdV	serology	70-90 %	(Adair et al., 1984)
wolves	Norway	canine adV	serology	68 %	(Akerstedt et al., 2010)
	Yellowstone NP, USA	canine adV	serology	94 %	(Almberg et al., 2009)

Table 3 continued.

2.3 Atadenovirus

2.3.1 Genus specific features

It has been recognized early on that the classification of AdV into only 2 genera, *Mastadenovirus* and *Aviadenovirus* might be inadequate and consequently avian, as well as bovine serotypes were divided into different (sub-) groups based on their biological properties (Bartha 1969, Zsak and Kisary 1984, McFerran *et al.* 2000). However, only in 1998, Benkö and Harrach published a proposal for a new genus, *Atadenovirus*, named after the strikingly high A+T content of the genomic DNA (Benkö *et al.* 1998, Dan *et al.* 1998). In search for the original host of *Atadenovirus*, AdV occurrence was investigated in a wide host range. As some atadenoviruses were detected in lower vertebrates, it was hypothesized that the original host of this genus might be squamate reptiles (*Squamata*). Hereby confirming that AdV have co-evolved with their host (Benkö *et al.* 2003). However, even though most known reptile AdV have been assigned to the genus *Atadenvirus*, the host range of atadenoviruses is not restricted to this specific vertebrate class. In fact, atadenoviruses have been detected in cattle(Dan *et al.* 1998), in goat (Lehmkuhl *et al.* 2001), in sheep (Barbezange *et al.* 2000, Both 2004), in mule deer (Zakhartchouk *et al.* 2002), in marsupial (Thomson *et al.* 2002), in birds (Hess *et al.* 1997) and in reptiles (Benkö *et al.* 2002, Farkas *et al.* 2002).

Differentiation criteria for atadenoviruses include presence of the genus-specific antigen, genus-specific genome organization or capsid protein complement. The genome length of atadenoviruses is between 29,576 bp and 33,213 bp and the G+C content between 33.6 and 43 %. While the central part of the genome is nearly similar to that of mastadenoviruses, the termini differ substantially. Among the multiple unique genes of atadenoviruses, are the gene coding for the structural protein p32K and LH3. LH3 forms the “knobs” on the virion surface and thus facilitats the structural differentiation of atadenoviruses. Some unique genes are only found in some genus members (Harrach *et al.* 2011). The consequence of the high A+T content of many atadenoviruses is unclear. It has been hypothesized that it might be advantageous in case of host jumps as it might facilitate the adaptation to the new host. Thus A+T rich AdV would represent AdV, which resulted from a recent interspecies transmission(Benkö *et al.* 2003). Another argument supporting this theory is that many atadenoviruses are not strictly host-specific and show higher virulence, than most mast- and aviadenoviruses (Benkö *et al.* 2003).

2.3.2 Epidemiology

Highly pathogenic atadenovirus infections in different reptile species have been reported from different regions of the world. The growing number of people housing snakes and lizards in captivity resulted in an increased import of these fragile animals. Many lizards died from an AdV outbreak during such an importation convoy. They were lethargic and anorexic and showed gastroenterocolitis (Ascher *et al.* 2013). In Queensland, a pathogenic Agamid atadenovirus infection in bearded dragons (*Pogona vitticeps*) was observed. They were presented with neurological signs, poor growth and occasional deaths (Doneley *et al.* 2014).

An example for a pathogenic avian atadenovirus is Duck AdV 1. It is widespread in ducks (Cha *et al.* 2013) and is transmitted from waterfowl to chickens, in which it causes the Egg-Drop syndrome (EDS)(Van Eck *et al.* 1976). EDS is characterized by decreased egg production, production of shellless, thin-shelled, discoloured or misshapen eggs in apparently healthy birds(Hess *et al.* 1997, Hafez 2010). Although the virus is considered to be apathogen in waterfowl, it has been associated with respiratory symptoms and tracheitis in ducklings and goslings (Ivanics *et al.* 2001, Brash *et al.* 2009, Cha *et al.* 2013). Thus far serological screening for antibodies against EDS was performed in several countries (Hafez 2010); among others, in the African countries, South Africa and Nigeria (Bragg *et al.* 1991, Durojaiye *et al.* 1991).

An atadenovirus was isolated from black-tailed deer with epizootic hemorrhagic disease in California (Lehmkuhl *et al.* 2001) and ovine atadenoviruses in lambs with mild to fatal pneumonia (DeBey *et al.* 2001). In deer infected with bovine AdV-5, symptoms of hemorrhagic enteritis were described (Woods *et al.* 1996). Bovine AdV-5 was also isolated from calves with polyarthritis, or “weak calf syndrome” (Coria *et al.* 1975, McClurkin and Coria 1975).

2.4 Siadenovirus

2.4.1 Genus specific features

Since a Pan-genera PCR targeting the polymerase gene has been established (Wellehan *et al.* 2004), siadenoviruses have been isolated from various animal species. As most of the siadenovirus types have been detected in birds (formerly Avian AdV Group II), the original hypothesis that siadenoviruses co-speciated with amphibians has been refuted. Characteristic for this genus is the biased G+C content and the leftmost gene, which is highly similar to the cellular sialidase encoded by certain bacterias (Davison *et al.* 2003).

Siadenoviruses have been isolated from psittacines (Wellehan *et al.* 2009), from a budgerigar (Katoh *et al.* 2009), from a great tit (*Parus major*)(Kovacs *et al.* 2010), from poultry (Pitcovski *et al.* 1998), from a South Polar skua (*Stercorarius maccormicki*) (Park *et al.* 2012), from birds of prey (Kovács *et al.* 2009, Kovacs *et al.* 2011), from tortoise (Rivera *et al.* 2009) and from a frog (Davison *et al.* 2000).

A novel siadenovirus was detected recently in Chinstrap penguins (*Pygoscelis antarctica*) from the Antarctica (Lee *et al.* 2014).

Siadenoviruses are distinguishable from other genera by serology on the basis of different genus-specific complement-fixing antigens, by phylogenetic analyses or on basis of their genomic organization. Among the adenoviridae family, siadenoviruses have the shortest genome: it is between 26, 163 bp and 26,283 bp and the G+C content between 34.9 and 38.5 %. As mentioned above, they possess a protein, which is related to sialidase and which is encoded by the left end of the genome. Besides this gene, there are others without homologous in any other genus (Harrach *et al.* 2011).

2.4.2 Epidemiology

Siadenovirus diseases with economic impact comprise the Haemorrhagic enteritis virus in turkeys (HEV), the Avian AdV Splenomegaly virus (AASV) in chickens and the Marble spleen disease virus (MSDV) in pheasants. All diseases are caused by three serologically and genetically indistinguishable isolates of Turkey AdV-3 (TAdV-3) (Pitcovski *et al.* 1998, Palya *et al.* 2007). In turkeys, HEV is characterized by intestinal hemorrhages and immunosuppression, but subclinical infections have also been observed (Palya *et al.* 2007). Chickens can be naturally infected with MSDV and HEV. Pathological findings of MSDV are pulmonary congestion, splenomegaly, hepatomegaly and congestion of egg follicles (Domermuth *et al.* 1982), whereas infection with HEV are mainly subclinical and only slight splenic and lung lesions can be revealed by pathological and histological analyses (Silim *et al.* 1978, Beasley and Clifton 1979, Veit *et al.* 1981). Chickens infected with HEV might represent a reservoir of infection for turkeys. In fact, in Japan, 50 % of the chickens were positive for HEV by agar-gel precipitation test (Yamaguchi *et al.* 1982), and in the USA, 46 % of the broiler-breeder chickens showed precipitation antibodies of HEV(Domermuth *et al.* 1980). In both countries the seroprevalence was age-related: positivity increased with age (Domermuth *et al.* 1980, Yamaguchi *et al.* 1982). It remains unclear if this high prevalence is due to persistent infection, as it has been shown for FAdV infections in chickens (Beach *et al.* 2009). Similar to FAdV, HEV is transmitted horizontally through direct or indirect contact (e.g. via contaminated manure) (Rautenschlein *et al.* 1998).

The Sulawesi tortoises (*Indotestudo forsteni*), in which siadenoviruses were detected, were in poor health and clinical signs described included anorexia, lethargy, mucosal ulcerations and palatine erosions of the oral cavity, diarrhea, and nasal and ocular discharge (Rivera *et al.* 2009).

Weight loss and lethargy, as well as leukocytosis and feather anomalies were reported for the two psittacine (*Psittacula cyanocephala and Cacatua alba*) in which the psittacine AdV-2 was detected. The clinical significance of this AdV remains however to be determined (Wellehan *et al.* 2009). In other naturally infected psittacines, siadenovirus infection was associated with immunsuppresion (depletion of lymphocytes in the spleen) (Gomez-Villamandos *et al.* 1995).

2.5 Aviadenovirus

2.5.1 Genus specific features

It is based on group-specific antigens, that the avian AdV were originally divided into 3 non-crossreacting groups (Group I corresponding to the genus *Aviadenovirus*, Group II to the genus *Siadenovirus* and Group III to the genus *Atadenovirus*). The formerly Group I avian AdV, comprises AdV which were detected from various avian species (McFerran *et al.* 2000). Besides the 5 FAdV species (FAdV A-E) detected in chickens, 3 other recognized species (Goose AdV A, Falcon AdV A and Turkey AdV B) belong to this genus and 2 more (Turkey AdV C and Turkey AdV D) are in discussion (Marek *et al.* 2013). More species might be added in future, as aviadenoviruse*s* have been detected in other bird species (e.g. pigeon, psittacine bird, turkey) (Raue *et al.* 2005, Schrenzel *et al.* 2005, Kajan *et al.* 2010). Complete genome sequences are available from members of every FAdV species (Chiocca *et al.* 1996, Ojkic and Nagy 2000, Kajan *et al.* 2010, Grgic *et al.* 2011, Griffin and Nagy 2011, Kajan *et al.* 2012, Marek *et al.* 2013, Marek *et al.* 2014, Marek *et al.* 2014).

By serology, *Aviadenovirus* are distinguishable from other genera on the basis of different genus-specific complement-fixing antigens. Other differentiation criteria are characteristic genome organization and phylogenetic distance. Besides the unique *Ichtadenovirus*, aviadenoviruses have the longest genome among the genera of the Adenoviridae family. The genome length of the completey sequenced aviadenovirus is between 43,804 bp and 45,667 bp and the G+C content between 53.8 and 66.9 %. While the central part of the genome is nearly similar to that of mastadenoviruses, the termini differ substantially (Harrach *et al.* 2011). In contrast to most other AdV, some species of the genus *Aviadenovirus* present two fiber proteins per vertex capsomer(Gelderblom *et al.* 1982). The completely sequenced FAdV A and FAdV C members have two fiber genes; completely sequenced FAdV B, FAdV D and FAdV E members in return have only a single fiber gene (Chiocca *et al.* 1996, Ojkic *et al.* 2000, Grgic *et al.* 2011, Griffin *et al.* 2011, Marek *et al.* 2012). Although TAdV-1 and Goose AdV-4 seem to possess two fiber genes (Kajan *et al.* 2010, Kajan *et al.* 2012), it remains unclear whether they possess two fibers per penton base.

Based on cross-neutralisation tests, the FAdV species have been further subdivided into 12 FAdV types (FAdV A-1; FAdV B-5; FAdV C-4 and -10; FAdV D-2, -3, -9, and 11; FAdV E-6, -7, -8a, and 8b) (Hess 2000). Until recently the European and the American system were incongruent. Now an international nomenclature system for FAdV has been instigated by the ICTV (Harrach *et al.* 2011). A subdivision into genotypes, based on phylogenetic analyses and well supported by bootstrap values has been suggested by Marek et al 2010 (FAdV A1, B1–B2, C1, D1–D4 and E1–E4). These genotypes correspond not entirely to the recognized serotypes (Marek *et al.* 2010).

Although different systems for the identification of FAdV types have been extensively studied, most are based on the analysis of the 4 hypervariable Loops (mainly L1) of the major capsid protein, the hexon protein, which is responsible for antigenic characteristics of AdV. Similarly to loops of the hexon proteins of mastadenoviruses *(Crawford-Miksza and Schnurr 1996)*, these 4 loops (L1 to L4) are type specific domains and protrude from the virus surface {except for L3 (Raue and Hess 1998)}. Thus phylogenetic analyses of partial hexon gene sequences are an adequate and quick method for within-genus analyses and for genotyping (Raue *et al.* 1998, Meulemans *et al.* 2001, Meulemans *et*

al. 2004, Ojkic *et al.* 2008, Mase *et al.* 2009, Marek *et al.* 2010, Lim *et al.* 2011). Apart from elaborate and expensive sequencing, other rapid and cost-effective alternative type and species identification methods have been proposed. With restriction fragment length polymorphism of the polymerase chain reaction product most of the FAdV types could be reliably identified (Raue *et al.* 1998, Meulemans *et al.* 2001, Meulemans *et al.* 2004, Raue *et al.* 2005). The application of real-time PCR facilitated not only detection and identification, but also quantification of FAdV (Romanova *et al.* 2009, Gunes *et al.* 2012). In other studies, real-time PCR and subsequent high resolution melting (HRM)-curve were proposed for the differentiation of the 12 FAdV serotypes (Steer *et al.* 2009, Marek *et al.* 2010, Dar *et al.* 2012). Combination of PCR and southern blot hybridization has been used to achieve a high sensitivity in type identification (Xie *et al.* 1999). Other methods, which have been proposed, are pyrosequencing (Pizzuto *et al.* 2010) and loop-mediated isothermal amplification(Xie *et al.* 2011).

Generally, the interpretation of serological tests, such as enzyme-linked immunosorbent assays, agar gel immunodiffusion and immunofluorescence, is impeded by the widespread presence of anti–FAdV antibodies in both, healthy and diseased birds (McFerran *et al.* 2000). Hence, contrary to PCR, these methods do not provide any information regarding active infection. Moreover, inconsistency between the types determined by serology or sequence analysis might be due to the fact that, even though the hexon gene is the main antigenic gene, other genes like the penton base and fiber also contribute to the serological properties (Hong *et al.* 2003, Kajan *et al.* 2013).

In the majority of the studies FAdV was detected with PCR in tissue samples of deceased poultry and the highest viral genome copy numbers was found in caecal tonsil (Romanova *et al.* 2009). Grafl et al, 2013 detected FAdV 1 also in cloacal swabs (Grafl *et al.* 2012). Although the virus load is higher in organs, cloacal swabs is an adapted material for avian AdV detection in live birds, especially as virus can be isolated in feces of infected animal for several weeks and is considered to be the main source of infection (Schade *et al.* 2013). Moreover, similar as to human (Garnett *et al.* 2009), AdV infections can become latent and reactivation with viral shedding might occur in situation of stress and immunosuppression (Girshick *et al.* 1980). Humoral immunity is theoretically type-specific and does not prevent AdV excretion, which is highest during the period of peak egg production (McFerran *et al.* 2000).

2.5.2 Epidemiology

FAdV are transmitted vertically, via the eggs, and horizontally, via oro-faecal route (Grgic *et al.* 2006, Grafl *et al.* 2012). Vertical transmission of FAdV is facilitated during the period of peak egg production, during which FAdV shedding is highest (McFerran *et al.* 2000). Furthermore, chickens could be infected with FAdV-1 by ocular inoculation in experimental settings (Okuda *et al.* 2001). Besides these direct transmission ways, indirect horizontal transmission, via fecally contaminated equipment, farm workers and insect or rodent vectors, plays a role for virus spread (Ono *et al.* 2007, Hafez 2010).

For most of the FAdV serotypes the pathogenicity is still questionable and correlational analysis did not reveal a definite association between AdV infection and specific pathology (Meulemans *et al.* 2001). Such analyses are complicated by the facts that FAdV have been isolated in asymptomatic chickens, and that often only some strains of a FAdV type are virulent, while others are not (Erny *et al.* 1991). Moreover, mixed infections with different FAdV serotypes or even FAdV belonging to different FAdV species have been repeatedly observed in the same bird (Kajan *et al.* 2013). For example while some FAdV-1 isolates have been associated with gizzard erosions in chickens, no pathogenic potential could be identified for other isolates (Okuda *et al.* 2001, Marek *et al.* 2010, Marek *et al.* 2010). Whether DNA sequence difference of TAdV-3 or PCR-RFLP analysis of the long fiber gene of FAdV-1 can be used to distinct different disease forms or between virulent and avirulent remains unclear (Okuda *et al.* 2006, Palya *et al.* 2007, Marek *et al.* 2010).

Some FAdV strains impair the humoral and the cellmediated immune function (Saifuddin and Wilks 1992, Singh *et al.* 2006, Schonewille *et al.* 2008). For instance, leukocytic depletion has been reported for FAdV-1, FAdV-4 and FAdV-8 infections (Saifuddin *et al.* 1992, Singh *et al.* 2006, Schonewille *et al.* 2008). Thus the question whether FAdV play a role as primary pathogens for chickens remains arguable and it poses a typical chickens-and-egg problem. In fact, on one hand, the compromised immunological capabilities favor secondary infections with pathogenic bacterial, viral, and fungal agents. On the other hand, infection with other viruses (e.g. IBDV and CAV), immunosuppression, mykotoxines and bad husbandry conditions increase the AdV virulence (Singh *et al.* 1996, Toro *et al.* 1999, Shivachandra *et al.* 2003). Besides immunosuppression, other factors increase the susceptibility of chickens for avian AdV induced disease and influence the virulence: age (Clemmer 1972) and genetic line (Beasley *et al.* 1979). Another consequence of the compromised immunological capabilities is the reduced quality of postvaccinal immunity. Thus it is of paramount importance that only healthy birds are vaccinated to ensure optimal immunity (Koncicki *et al.* 2006).

In cases where FAdV cause severe diseases with negative impact on egg and meat production, strain identification is of importance e.g. for specific vaccine development. The high pathogenicity of several AdV can be possibly explained by a recent host switch to an unadapted host (Benkö *et al.* 2003). This hypothesis was also generated for highly pathogenic FAdV and was well-supported by phylogenetic analyses (Marek *et al.* 2013). One example is adenoviral **inclusion body hepatitis (IBH)**, which has been associated with different isolates of FAdV species D, E and A (Ramis *et al.* 1992, Hess 2000). In Canada, FAdV serotypes 1 (CELO virus), 2, 4, 8a and 11 were detected in chickens with IBH (Ojkic *et al.* 2008), whereas in Slowenia the FAdV serotype 8b (Zadravec *et al.* 2013), in Chile FAdV serotype 4 (Toro *et al.* 1999) and in Japan serotype 2 (Nakamura *et al.* 2011, Mase *et al.* 2012) were identified as causative agent of IBH. In Northern India, FAdV serotypes 1, 6, 7 and 8 (Singh *et al.* 1996, Singh *et al.* 2002), in Australia serotypes 8b and 11 (Steer *et al.* 2011) and in Iran FAdV-11 were isolated from chickens with IBH (Hosseini and Morshed 2014). Diseased chickens, but also other bird species show mainly mild symptoms, as for example ruffled feathers. But the disease can be mortal (mortality between 5-30 %) for breeders, between three and seven weeks of age(McFerran *et al.* 2000).

Symptoms characteristic for **hydropericardium syndrome** (HPS, Angara Disease or Litchi heart disease) in chickens and other bird species (e.g. quails, pigeons and wild black kites) are identical to IBH, but with additional hydropericardium and higher mortality rate (McFerran *et al.* 2000). If both,

IBH and HPS, are observed in the same bird, the resulting disease is referred to as hepatitis hydropericardium syndrome (HHS) (Hafez 2010, Palanivelu *et al.* 2014). This emerging immunosuppressive disease has been predominantly described in broilers (Hussain *et al.* 2012, Palanivelu *et al.* 2014). It is characterized by rapid onset, high mortality (up to 60 %), anemia, necrotic hepatitis and the pathognomonic hydropericardium (Kataria *et al.* 2014, Palanivelu *et al.* 2014). Serotypes FAdV-4 (FAdV C) and FAdV-8 (FAdV E) were isolated in chickens with hydropericardium in several Asian (India, Iraq, Kuwait, Pakistan, Bangladesh, Korea and Japan), American (Mexiko, Peru, Ecuador and Chile) and European (Slovakia, Greece, Russia) countries (Erny *et al.* 1991, Mazaheri *et al.* 1998, Hess *et al.* 1999, Mase *et al.* 2009, Hafez 2010, Kataria *et al.* 2014).

Another FAdV induced disease was described in several European and Asian countries: FAdV-1 was isolated from chickens with the pathomorphological lesions of **gizzard erosions** (blood in the gizzard and necrosis below the keratinoid layer)(Okuda *et al.* 2001, Ono *et al.* 2003, Marek *et al.* 2010, Domanska-Blicharz *et al.* 2011, Lim *et al.* 2011, Grafl *et al.* 2012, Gunes *et al.* 2012, Schade *et al.* 2013).

As the FAdV strain ‘‘8844/2010 Debrecen’’ was isolated from the Achilles tendon of a chickens (Kajan *et al.* 2011), it has been hypothesized that FAdV may be involved in the pathomechanism of tenosynovitis in chickens (Kajan *et al.* 2013). Other symptoms related to FAdV infections include pneumonia and tracheitis (Crespo *et al.* 1998), proventriculitis (Guy *et al.* 2005) and pancreatitis (Grimes *et al.* 1977). Moreover decreased feed intake and poor growth have been described in infected chickens (Clemmer 1972, Adair and Smyth 2008).

Symptomatic aviadenovirus infections are not restricted to Galliformes. Goose AdV cause hepatitis and hydropericardium syndrome in young goslings (Ivanics *et al.* 2010) and AdV could also be detected in young goslings with inclusion body hepatitis (Riddell 1984). Besides, recently a pathogenic AdV (new-type gosling viral enteritis virus, NGVEV) was defined as causative agent of new type gosling viral enteritis. Characteristic for the infection are somnolent goslings with diarrhea, neurological symptoms and sudden death (Cheng *et al.* 2001, Chen *et al.* 2009, Chen *et al.* 2010, Chen *et al.* 2011).

2.6 Emerging infectious diseases and risk factors for zoonotic transmission

Modification to natural ecosytems, changes in agricultural practices and globalization of trade are among the major drivers of emerging infectious diseases (EID) (Wolfe *et al.* 2005). These drivers change the host and pathogen ecology by altering transmission and exposure patterns and can result in the introduction of exotic pathogens (=pathogen pollution or human-mediated pathogen invasion) (Daszak *et al.* 2000). “EID are as yet unrecognized infections, or previously recognized infections that have expanded into a new ecological niche or geographical zone and are often accompanied by a significant change in pathogenicity”(Morse and Hughes 1996).

The majority of these EIDs are zoonoses, which ‘’are diseases or infections naturally transmitted between vertebrates and humans’’ (World Health Organization) and which represent more than 60 % of the EID (Morens *et al.* 2004, Jones *et al.* 2008). Examples for such zoonotic pathogens are leptospirosis, echinococcosis, toxoplasmosis, anthrax, brucellosis, rabies, Q-fever, type A influenzas, severe acute respira tory syndrome (SARS), Ebola haemorrhagic fever, and HIV. Parrish et al, 2008 described the three necessary steps for successful host switch and viral disease emergence: “(1) initial single infection of a new host with no onward transmission (spillovers into “dead-end” hosts), (2) spillovers that go on to cause local chains of transmission in the new host population, before epidemic fade-out (outbreaks), and (3) epidemic or sustained endemic host-to-host disease transmission in the new host population” {or species jump (Flanagan *et al.* 2012)} (Parrish *et al.* 2008). For successful species jumps (=step 3), contrary to simple spill-over events (=step 1), mutation of the virus is a prerequisite. Such mutations enable the virus to adapt to the new host and to be efficiently transmitted among the new host population (Flanagan *et al.* 2012). Favorable environment characteristics (e.g. high prevalence of pathogen, high rate of exposure) are essential for pathogen transmission and favorable pathogen and host characteristics are essential for efficient infection and disease development.

Viruses represent a large proportion of the zoonotic pathogens. Due to the high mutation rates, RNA viruses can rapidly adapt to new hosts, and are the major virus class involved in EID (Cleaveland *et al.* 2001). For instance, if the mutations include the proteins implicated in cell-binding, viruses might evolve the ability to bind to a receptor in a novel target host species. Moreover, such modifications of the viral genomes provide viruses the potential to react to host immunity, to variations in vector occurrence and in host ecology (Howard *et al.* 2012). The reproductive ratio (*R*0) has been defined as measure for the pathogen’s ability to cause an outbreak and is defined as the number of secondary cases in a population caused by a single case, assuming that all other members are susceptible. If *R*0 of a pathogen is >1, it is likely to cause an outbreak and if *R*0 of a pathogenis <1, it is likely to be eliminated (Heesterbeek 2002).

From 1996 to 2009, 53 % of the EID have arisen in Africa (Chan *et al.* 2010). Thus this continent is considered to be the main hot-spot for EID (Jones *et al.* 2008). Over the past decades, the intensification and expansion of land-use increased the wildlife-livestock-human interaction. In fact this change in land-use resulted not only in altered distribution and abundance of the host species, but also intensified the human-wildlife-and domestic animal interface (Schrag and Wiener 1995). The majority of the EID in humans originated from wildlife (Jones *et al.* 2008) and for many EID bats and rodents played a major role as reservoir and vector for pathogen spread (e.g. rabies and SARS) (Wood *et al.* 2012, Luis *et al.* 2013). Besides the close interspecies contact, development of ecotourism, bushmeat trade, translocation of live domestic and wild animals (Chomel *et al.* 2007) and global warming (Howard *et al.* 2012) are other risk factors for zoonotic transmission. A consequence of the partially anthropogenic global warming can be altered vector distributions and thus the increased occurence of vector-borne diseases (Howard *et al.* 2012).

The augmented exposure to novel pathogens is also due to augmented magnitude of bushmeat hunting in many regions of the world (the so called bushmeat crisis) (Bushmeat Crisis Task Force 2000, Gessain *et al.* 2013). “Wildlife and the meat derived from it is referred to as ‘bushmeat’ (in French-viande de brousse)”(Bushmeat Crisis Task Force 2000). 90 % of the worldwide consumed

bushmeat is extracted from African forests (Malhi *et al.* 2013). The increasing urban and global demand for bushmeat could only be satisfied by a parallel increasing bushmeat trade between the villages and the urban areas. This evolution was alleviated by easier access to non-human primate (NHP) habitats via recently built logging roads and an expanding human population (Wilkie *et al.* 1992, Wolfe *et al.* 2004). Several studies report the high risk for zoonotic transmission of viral pathogens during bushmeat hunting and other activities related to bushmeat (Peeters *et al.* 2002, Wolfe *et al.* 2004, Wolfe *et al.* 2005, Ndembi *et al.* 2009, Smith *et al.* 2012). Hence there are numerous examples for zoonotic or cross-species transmission of pathogens during bushmeat handling, particulary when handling non-human primate (NHP) carcasses. In fact, close phylogenetic relationship facilitates pathogen exchange between closely related species, such as human and NHP(Davies and Pedersen 2008). The risk is highest during close contact to great apes (e.g. chimpanzee, gorilla) (Calattini *et al.* 2007, Mouinga-Ondeme *et al.* 2012). The best-known examples are the *siman Immunodeficiency Virus (SIV) and the simian T-Lymphotropic Virus (STLV),* SIV-1 and STLV-1, which crossed the species barrier on multiple occasision and mutated to the *human Immunodeficiency Virus (HIV) and the human T-Lymphotropic Virus (HTLV)* (Hahn *et al.* 2000, Peeters *et al.* 2002, Wolfe *et al.* 2005). Individuals reporting direct contact to NHP (e.g. bushmeat or pets) have been tested positive for Simian Foamy Virus (SFV) under natural conditions (Wolfe *et al.* 2004, Mouinga-Ondeme *et al.* 2012). Regions, which are habitat of sooty mangabeys, overlap with regions, where HIV-2 is endemic in humans (Hahn *et al.* 2000). Additionally, a recent study on HTLV in the Taï region showed that the majority of the HTLV-1 infections in the local population originated directly from a NHP host and that human-to-human transmission occurs, but is rare (Calvignac-Spencer *et al.* 2012). Moreover, herpesvirus-B, primate malaria and tuberculosis from captive NHP have been detected in caring humans (Ndembi *et al.* 2009). Besides evolutionary relatedness, elevated rate and intensity of contact further increase the risk for pathogen transmission (Parrish *et al.* 2008).

Epidemiological analysis of the risk factors for simian foamy virus (SFV) revealed that the major risk factors were “apes” and “bites” (Calattini *et al.* 2007, Betsem *et al.* 2011). It was concluded that every ape-related activity, during which humans are at risk of getting bitten are high-risk occupations for the zoonotic transmission of (blood-borne) pathogens (e.g. SFV, SIV, and STLV) (Betsem *et al.* 2011). Every injury (e.g. bite or scratch) inflicted by an infected NHP (including chimpanzee, mandrill, baboon, and cercopithecus, colobus and cercocebus species) can lead to an infection in humans (Betsem *et al.* 2011). Hunting and ape bites were also identified as high risk factors in the study on cross-species transmission of SFV in Gabon (Mouinga-Ondeme *et al.* 2012). In Cameroon, more than 35 % of the hunters with a history of injury by a NHP were SFV infected (Calattini *et al.* 2007). Contact to live NHP is certainly mainly restricted to tropical Africa and Asia, but NHP bushmeat plays a role for the rest of the world. In fact, illegal bushmeat importation might threaten animal and human health worldwide as they might be vectors of various wildlife zoonoses (e.g. retroviruses and herpesviruses) (Smith *et al.* 2012, Falk *et al.* 2013).

Thus far, it cannot be foreclosed that transmission ways other than NHP bites might play a role for humans. In fact, in Cameroon, one woman was infected with SFV after solely handling bushmeat (Calattini *et al.* 2007) and SFV was also detected in persons who worked with chimpanzees or baboons, but had never been injured (Switzer *et al.* 2004). SFV can be transmitted horizontally between different NHP species, via sole exposure to saliva, in which a high concentration of SFV was found (Falcone *et al.* 1999, Murray *et al.* 2006). While in a natural hunter-prey system, interspecies

transmission of SFV and STLV from colobus monkey to chimpanzees occurs (Leendertz *et al.* 2008), interspecies transmissions of SIV, Partetraviruses or Herpesvirus have not been observed (Leendertz *et al.* 2011, Adlhoch *et al.* 2012, Murthy *et al.* 2013). However, interspecies transmission of SIVsm from captive sooty mangabey to different macaque species has been reported (Hahn *et al.* 2000). Although in studies on SFV, NHP pets were not considered to represent a major risk, as they are mostly orphans, are separated from any potential infection source and seldomly bite since they are well habituated (Wolfe *et al.* 2004, Betsem *et al.* 2011, Mouinga-Ondeme *et al.* 2012), they might play a role for the zoonotic transmission of other pathogens (Chomel *et al.* 2007).

Besides wildlife, domestic animals might also be an important pathogen source for humans. The majority of the pathogens of domestic animals are multiple host pathogens (e.g. >90 % of the pathogens from domestic carnivores) and many of them have zoonotic potential (e.g. >40 % of the pathogens from domestic carnivores) (Cleaveland *et al.* 2001). Examples are Trypanosomas cruzi (chagas disease), Giardia and Echinococcus multilocularis (Ryan and Caccio 2013, Thompson 2013). Wildlife pathogens play also a role for domestic animals. Interspecies transmission of trichinella has been reported in domestic dogs from sub-saharan Africa, consuming meat from infected bush pigs (Mukaratirwa *et al.* 2013). Such an introduction of pathogens from the sylvatic to the domestic circle is favored in areas, where human and wildlife habitat overlap (e.g. through shared water-and food reservoir) and where the specific vectors naturally occurs (e.g. culicoides, moskitos). A consequence can be the reemergence of zoonotic diseases, which had been controlled in the domestic animal reservoir (Chomel *et al.* 2007).

Although, research mainly focuses on zoonotic transmission of pathogens from animals to humans, a reverse transmission from humans to animals is not infrequent and can have dramatic consequences. Risk factors for such “zooanthroponoses”, “anthropozoonoses” or “reverse zoonoses” mainly equal the above mentioned risk factors for zoonotic disease transmission (Epstein *et al.* 2009). To avoid misunderstandings in the present study, we chose to use “zoonoses” for diseases where the source is an infectious animal and “anthropozoonoses” for disease, where the source is an infectious human. Thus far, the majority of the studies investigating reverse zoonotic transmission focused on bacterial or mycobacterial infections and on cross-species transmission of viral agents between humans and NHP (Köndgen *et al.* 2008, Morgan 2008, Messenger *et al.* 2014). However cross-species transmission of the neglected viral infections between livestock and humans might have significant health and economic consequences (Messenger *et al.* 2014).

Different aspects are important for combating EID and zoonotic diseases. First, global epidemiological surveillance of high-risk pathogens is necessary, particulary in EID hotspots (Chan *et al.* 2010, Howard *et al.* 2012). Further, analysis of the pathogen dynamics is essential to understand the process of emergence (Wolfe *et al.* 2005).The principle of the One Health approach is to unite veterinarians, occupational health physicians and public health operators in the battle against EID (Rabozzi *et al.* 2012). The One Health triad comprises human and animal health and considers the changing ecosystems of their habitats (Thompson 2013). Hence instigation of such an integrated human-animal health system would be beneficial for humans and animals, particularly as it has been shown to be more cost-effective and efficient than conventional health systems (Zinsstag *et al.* 2009).

2.7 Evolution and zoonotic potential and cross-species transmission of Adenoviruses

In general, AdV appear to have undergone host-dependent evolution and hence to have co-evolved with their respective vertebrate host class (Benkö *et al.* 2003, Davison *et al.* 2003). This hypothesis of codivergence of AdV with their host is widely recognized and is supported by many AdV characteristics, which are typical for DNA viruses: their complexity, large size and slow rate of evolution. Moreover, the divergence of most viral lineages of the AdV species virtually mirrors the divergence of their vertebrate host (Benkö *et al.* 2003, Davison *et al.* 2003). However, although many findings on AdV host spectrum and evolution suggest that the coevolution hypothesis is applicable to most of the presently known AdV, many evidence for host switches have been discovered. In fact, if AdV had strictly co-evolved with their host, it would be possible to assign a specific vertebrate class to every genus. This is feasible for *Mast-*, *Avi-* and thus far *Ichtadenovirus*, but not for *Atadenovirus* and *Siadenovirus*.

The phylogenetic relationship among atadenoviruses does not reflect the relationship among the host species and multiple host transfers of atadenoviruses from reptiles to birds, marsupial mammals and ruminant mammals might explain the wide host spectrum of this genus (Harrach 2000). There are many hints suggesting that these host transfers happened recently. The first is the well conserved genome organization among all atadenoviruses, no matter what vertebrate class they have been detected in: bird, mammal or reptile. Moreover squamate AdV form an ancestral and monophyletic clade within the genus and hence one can assume that birds and ruminants acquired atadenoviruses directly from squamate hosts (Doszpoly *et al.* 2013); albeit, this monophyly was not confirmed by all phylogenetic analyses (Ascher *et al.* 2013). Another critical hint is the biased nucleotide composition of atadenoviruses in all hosts with exception of the squamate host. In bacteria, G+C in contrast to A+T rich gene regions have been associated with increased genomic stability, increased resistance to homologous recombination (Gruss *et al.* 1991) and higher growth rates(Raghavan *et al.* 2012). Similarly to *Atadenovirus*, biased AT composition has been described for *Siadenovirus* (Wellehan *et al.* 2009) and no vertebrate class could be convincingly assigned to this genus, regrouping AdV from bird, amphibian and reptile host (Harrach *et al.* 2011). Although amphibians were first hypothesized to be the evolutionary origin of this AdV lineage, the majority of the presently known members of the genus Siadenovirus have been detected in birds (Benkö *et al.* 2003, Davison *et al.* 2003, Katoh *et al.* 2009, Kovacs *et al.* 2010, Park *et al.* 2012). As several siadenovirus types have been isolated from distantly related host species, one can assume that this genus is less host-specific (Wellehan *et al.* 2009).

Other lines of evidence suggest that occasional host transfers do occur in the virus family. Phylogenetic analyses of the human and simian AdV (HAdV and SAdV) revealed several hints that cross-species transmission of HAdV and SAdV occured. Particularly the HAdV from species B, E and G might have evolved from their simian counterpart and originated in NHP. Not only the fact that more simian than human AdV belong to these species (e.g. HAdV E), but also investigation of the evolutionary process of HAdV and SAdV yielded indications that HAdV B originated from gorillas and HAdV E from chimpanzees (Calcedo *et al.* 2009, Roy *et al.* 2009, Wevers *et al.* 2011, Duncan *et al.* 2013). The only member of species E, which has been obtained from a human, HAdV 4, contains a recombinant genome comprising sequences of human and simian AdV (Purkayastha *et al.* 2005,

Dehghan *et al.* 2013). The sole HAdV G type, HAdV-52, from a human was detected in a patient with gastroenteritis and is closely related to SAdV-1 and SAdV-7 (Jones *et al.* 2007). Moreover the facts that asymptomatic rhesus macaques frequently shed AdV, which are closely related to HAdV (Roy *et al.* 2012) and that wild great apes seem to shed AdV belonging to every HAdV species (Wevers *et al.* 2011) suggest that zoonotic transmission occurred during AdV evolution. Taken together, the phylogenetic and recombination analyses of HAdV and SAdV showed clear evidence for past cross-species transmission, but no statements can be made on the direction of this transmission.

Evident zoonotic transmission of SAdV occurred in the California National Primate Research Center. Here, a novel titi monkey AdV was identified during a deadly outbreak in New World monkeys (*Callicebus cupreus*) and in a diseased researcher in close contact with the captive monkeys (Chen *et al.* 2011). This same AdV induced respiratory symptoms in experimentally infected marmosets (*Callithrix jacchus*) (Yu *et al.* 2013). Another evidence for zoonotic transmission of AdV was a SAdV C, causing respiratory illness in captive baboons (*Papio hamadryas anubis*) and flu-like symptoms in a human researcher (Chen *et al.* 2011, Chiu *et al.* 2013). Thus strict precaution measures when handling non-human primates should be implemented to prevent zoonotic transmission of AdV, particularly as high prevalence of AdV shedding has been observed in asymptomatic non-human primates (Roy *et al.* 2009).

The zoonotic potential of AdV seems to be restricted not only to an animal-to-human pathogen transmission: there is strong evidence suggesting that reverse transmission of AdV occurs. Antibodies against simian and canine AdV have been detected in human sera and vice-versa, anti-HAdV antibodies in animal sera (Paillard 1997, Kremer *et al.* 2000, Xiang *et al.* 2006). For example in a study from the USA, 44 % of the captive chimpanzees had antibodies to human AdV-5 and in sub-saharan Africa a high prevalence of neutralizing antibodies to chimpanzee AdV was found in humans (Xiang *et al.* 2006). AdV, closely related to feline AdV have been isolated in children suffering from respiratory and gastrointestinal symptoms (Phan *et al.* 2006, Luiz *et al.* 2010), suggesting that HAdV may be transmitted from felines to humans. In experimental settings, other evidences for anthropozoonotic transmssion of AdV were found. HAdV can infect cells of virtually all mammalian species, but late genes are seldomly expressed (Horwitz *et al.* 2007). Jogler et al, 2006 tested cell cultures from seven animal species and described in porcine cells, productive replication of HAdV-5, comparable to human cells. Cell lines from other species seemed to be permissive for HAdV-5, but at reduced efficacy. Moreover an interstitial pneumonia was induced in a swine experimentally infected with HAdV-5 (Jogler *et al.* 2006). A bovine AdV closely related to HAdV was detected in cattle (Klein *et al.* 1960). HAdV inoculated in rodents resulted in the development of different tumors (Kosulin *et al.* 2007, Kosulin *et al.* 2013) and HAdV-36 was associated with obesity in rhesus monkeys, chickens, mice and non-human primates (marmosets) (Dhurandhar *et al.* 2000, Dhurandhar *et al.* 2002). Vaccines based on human AdV have been successfully tested in different animal species, where they induced a good immune response. Consequently, it was hypothesized that replication of the HAdV vector might occur to some extent in the animal host (Prevec *et al.* 1989).

Additionally, numerous evidences for cross-species transmission of animal AdV have been reported. Sheep, deer and camelids can be naturally infected with bovine AdV, which may even play a role in enteric and respiratory tract disease (Belák and Pálfi 1974, Horner and Read 1982, Barbezange *et al.* 2000, Lehmkuhl *et al.* 2008, Intisar *et al.* 2010). Ruminant AdV are phylogenetically closely related

and in reciprocal cross-neutralization tests cross-reactions between ovine, caprine and bovine AdV have been described (Adair *et al.* 1982). Similarly canine AdVs can infect and cause severe disease in several domestic and wild carnivore species (e.g. bears, wolves, raccoons, and sea lions) and in pandas (Qin *et al.* 2010). Moreover, canine AdV seems to replicate in goats following experimental inoculation, but is not shed through feces and urine (Qin *et al.* 2012). Furthermore, identical or nearly identical siadenovirus sequences have been isolated from distantly related snake and squamate species (Ascher *et al.* 2013) and the atadenovirus, Duck AdV- 1, can infect different bird species with varying pathogenicity (Durojaiye *et al.* 1991, Hafez 2010, Cha *et al.* 2013). The close genetic relationship between canine AdV and bat AdV evoked the hypothesis of a past interspecies transmission and of a bad origin of the canine AdV. Arguments for the past host switch, were the relatively high pathogenicity and broad host range of canine AdV (Li *et al.* 2010, Janoska *et al.* 2011, Kohl *et al.* 2012). Phylogenetic reconstructions also revealed a close relationship between equine AdV-1 and bat AdV(Cavanagh *et al.* 2012). Thus it is conceivable that equine, bat and canine AdV may share a common ancestor. Another evidence for a wide host range of bat AdV is susceptibility of many animal and human cell lines to bat AdV (Li *et al.* 2010).

Thus far, no definite statements can be made on AdV evolution as phylogenetic calculations are still unrooted, due to the lacking data on the most ancestral genus. To fill this knowledge gap, the only fish AdV is principally used as outgroup, but the current interpretation of AdV evolution might change substantially if an even more ancient clade was detected. It has been hypothesized that the most recent ancestor of all modern AdV might have been an AdV that existed before the divergence. of bony fish from other vertebrates (Davison *et al.* 2003).

2.8 Recombination of Adenovirus

Recombination has been defined as the horizontal exchange via conjugation, transformation, or transduction of genetic material: homologous recombination being the exchange of related gene sequences and nonhomologous recombination the exchange of nonrelated gene sequences. Recombination has been recognized as major mechanism for virus evolution, leading to the high diversity observed amongst viruses (Posada *et al.* 2002). Recombinant viruses often show an expanded host range, altered virulence and modifed tissue tropism. However many recombination events give rise to deleterious non-infectious viruses (Parrish *et al.* 2008).

A prerequisite for natural recombination is frequent co-infection, which is more likely for viruses developing persistent infections. AdV meet these requirements and homologous recombination has been commonly observed for AdV (Crawford-Miksza *et al.* 1996, Lukashev *et al.* 2008). It has even been proposed for HAdV D evolution that recombination might play a greater role than base substitution (Robinson *et al.* 2013). Although under natural circumstances, recombination events between members of the same species predominate, recombination events between AdV of distinct species occur.

Among the HAdV, HAdV D are particularly prone to homologous recombination (Robinson *et al.* 2009, Yang *et al.* 2009, Kaneko *et al.* 2011, Liu *et al.* 2011, Liu *et al.* 2012, Robinson *et al.* 2013, Singh

et al. 2013), and HAdV D recombinants have been associated with pathogenicity (Walsh *et al.* 2009, Robinson *et al.* 2011, Robinson *et al.* 2011, Matsushima *et al.* 2013, Singh *et al.* 2013). The virulent type D53, a recombinant between D8, D22, D37 and at least one unknown HAdV D type, showed a modified tropism and induced inflammation of the cornea (Walsh *et al.* 2009). Another recombinant type, D56, was involved in fatal pneumonia in a neonate and keratoconjunctivitis in three adults (Robinson *et al.* 2011). Modification of tissue tropism through recombination has been described for HAdV D60 (Robinson *et al.* 2013). Besides for HAdV D, recombination events have been observed between certain other HAdV species (Ebner *et al.* 2005, Robinson *et al.* 2009, Walsh *et al.* 2009, Yang *et al.* 2009, Walsh *et al.* 2010, Kaneko *et al.* 2011, Walsh *et al.* 2011, Dehghan *et al.* 2013). A recent example is HAdV-B35, the causative agent of an acute respiratory disease (ARD) outbreak in China, and which contains a partial hexon recombination between HAdV-B14 and -B11 (Walsh *et al.* 2010).

On several occasions, AdV recombinants between human and simian AdV have been reported. Recombination is facilitated by the facts that both, human and simian AdV, can infect humans and NHP and that both are closely related. One example is the simian AdV-ch1, which was isolated from a chimpanzee in China. Recombination analysis revealed a potential recombination event between this simian AdV and a human AdV (Zhou *et al.* 2014). The only member of species E, which has been obtained from a human, HAdV-4, contains a recombinant genome comprising sequences of human and simian AdV (Dehghan *et al.* 2013). An evidence for lateral DNA transfer between different simian and human AdV was also suggested for the SAdV-35 (Dehghan *et al.* 2013). Lateral gene transfers have also been described between different SAdV types, isolated in non-human primates (Roy *et al.* 2009).

Data on recombination between other animal AdV are scarce. On several occasions, coinfection of different AdV strains (from different genera) were detected in a same animal individual (Balboni *et al.* 2013, Kajan *et al.* 2013), however only in few studies recombination analyses were performed and thus the true extent of natural recombination among AdV infecting animals remains obscur. There is evidence that the porcine AdV-5 resulted of a recombination event between a mastadenovirus and an atadenovirus. This would be the first decribed recombination event between AdV from different genera. While the sequence of the tail and shaft of the fiber gene corresponded to that of mastadenoviruses, the sequence of the fiber knob corresponded to that of atadenoviruses. In depth recombination analyses were unfeasible in that study due to the limited Genbank dataset (Nagy *et al.* 2002). Similarly, the sequence of the fiber knob of Duck AdV-1 corresponds to that of aviadenoviruses, whereas the remaining sequence is typical for atadenoviruses. Hence it has been suggested that the fiber gene might be particularly prone to recombination (Harrach *et al.* 2011). Hints for potential recombination were identified for ovine AdV, porcine AdV, and bovine AdV, but the parent sequences were not determined (Sibley *et al.* 2011).

The genome of HAdV D, but also of other HAdV, presents several hypervariable gene regions, located mainly in the genome regions coding for surface epitopes (hexon, penton base, and fiber) and for immune modulatory proteins (E3) (Robinson *et al.* 2013). Homologous recombination of such gene region might provide advantages for the circumvention of the immune system of the host (Walsh *et al.* 2010, Robinson *et al.* 2013). Moreover as these hot-spots for recombination overlap with the target sites of serum neutralization and of several PCR systems, type identification based on serology

or blast analysis of partial genomes can be misleading. Only analysis of whole genome sequences can provide absolute certainty about the respective HAdV type (Singh *et al.* 2012).

3 Material and Methods

3.1 Study region

The Taï region in South Western Côte d`Ivoire is situated between the Taï National Park, the largest tropical rain forest of Western Africa, and the Cavally River, which runs along the border to Liberia *(Figure 2 and Supplementary Figure 1)*. The local climate is sub-equatorial and the mean temperature is of approximately 25°C, the relative humidity of approximately 85 %. The Taï National Park (TNP) expands over 5.360 km² (N'Zo Fauna Reserve included) and is situated between 5°15'-6°7'N and 7°25'-7°54'W. The park is habitat to many endangered species, as for example Pygmy Hippopotamus (*Hexaprotodon liberiensis)*, Chimpanzees (*Pan troglodytes verus*) and Jentink`s Duikers (*Cephalophus jentinki)* (http://www.parcnationaltai.com/index.php/fr/presentation/historique.html). Although this tropical forest is a recognized national park since 1972 and even an UNESCO World Heritage Site since 1982, a dramatic decrease in biodiversity has been observed in the last decades as a direct consequence of the population growth and thus increasing human pressure on the park (Campbell *et al.* 2008, Campbell *et al.* 2011, N'Goran *et al.* 2013, WCF 2013). Furthermore, the political instability led to an interruption of the local control measures (e.g. by the OIPR, Office Ivoirien des Parcs et Réserves) for the protection of the National Parks and many zones of the protected parks are still illegally cultivated and inhabitated ("zone d'occupation controlée", ZOC) (UICN/BRAO 2008, Koné 2013, International Crisis Group 2014, Office lvoirien des Parcs et Réserves (OIPR) 2014). Illegal bushmeat hunting has reached alarming amplitude, as bushmeat hunting for subsistence has been partially replaced by the more profitable commercial hunting {bushmeat being more valuable than domestic meat (Vega *et al.* 2013)} (Refisch 2005). It has been estimated that 9178 duikers and 4363 monkeys are consumed per year by families and in restaurants in Taï, Zagne and Djouroutou (latters are villages situated South to Taï village) (WCF 2013). The NGO Wild Chimpanzee Foundatioun (WCF) works in the region "to enhance the survival of the remaining wild chimpanzee populations and their habitat, the tropical rain forest, throughout tropical Africa" (http://www.wildchimps.org/wcf/english/files/mission.htm). With diverse activities in the Taï region (e.g. awareness compaigns, or environmental education of schoolchildren etc.), the WCF attempts to sensibilize the local population for the preservation of chimpanzees and of the forest.

The people living at the park boundary are predominantly subsistence hunters, pastoralists and cultivators. Many rear livestock (mainly ruminants, chickens and pigs) for personal consumption, but also as potential cash reserve or as store of wealth and insurance. Thus animal health directly influences human health, since the loss of an animal entails not only loss of protein provision, but also of the cash reserve required in emergency situations (e.g. medical conditions). Most of the animals roam freely through the villages, feed on human waste and leftovers and often share water supply with the local population *(Figure 2)*. On several occasions they have been observed within the human habitation and next to the cooking area. There is no clear separation between cooking, cleaning, washing and slaughtering area. During the days, cows and on occasion also small ruminant herds are moved by the farmers in search of fresh pasture and water. In several villages, animals are confined overnight in simple pens or enclosures built from local materials. Especially young piglets are regularly kept in small sheds during fattening. Some keep multiple animal species in the same restricted area for commercial purposes. Dogs in these rural communities serve primarily as hunting

animals or as protection for the properties. Most of these animals most likely have never received any vaccinations or primary health care. Cocoa and rubber cultivation and sale is the main income source in this fertile region. Together with the Guémon region, it is one of the major producer regions and until now one of the most unstable and undeveloped regions of Côte d`Ivoire (the world`s largest cocoa producer) (International Crisis Group 2014). Despite the fragile security situation since the political crisis after the presidential election in 2010 and Liberia`s civil war until 1996, people from the neighboring countries, but also from other parts of CI immigrated to the region, hoping to attain wealth through the cocoa and rubber trade (approx. 45 % of small holders are foreigners) (International Crisis Group 2014). Land transactions are merely unregulated and "Might is right"is the rule (International Crisis Group 2014) and hence the amount of rubber and cocoa plantations has grown in parallel with the human population (Brou 2009). This agriculture development led to an increasing extent of deforestation (Brou 2009, Koné 2013) and hence of human encroachment into the tropical ecosystem. Only an earth road connects the villages to the next larger town, Guiglo (at about 40 km of Taï village) and the cell phone network is the sole direct communication way. In some larger villages (e.g. Taï) electrical power is sporadically provided by a generator and current water is available on restricted hours a day. Other villages have neither a regular electricity, nor current water supply. In these villages washing, drinking and cooking water is obtained from wells. Many of these wells have no cover and are thus not protected from contamination through animals and rain water. The external edge of many wells is nearly at ground-level. This increases the contamination risk during rainy season, when the strong rains carry everything away and might flood the wells. In several villages, the European Union supported the construction of well-maintained wells with a manual pump, and a fix cover. Moreover the well water is theroretically regularly tested and treated and the amount of extracted water controlled. However discussion with several locals revealed that this water treatment is only rarely performed and the application of the chemical substances is often inexact. Furthermore, since the amount of these wells is still insufficient, people often still extract too much water despite the regulations. During the sampling mission in 2012, the ONUCI (United Nations Operation in Côte d`Ivoire) provided supplementary drinking water provisions, mainly to support the increasing number of refugees, which arrived in the region as a result of the crisis. Moreover the NGO "Croix Rouge" was building wells and water towers in several villages in the south of Taï village.

The only hospital in the region is located in Taï village. Besides this hospital, well-managed "cases de santé", or health centers, can be found in Ponan, Keibly, Gahably and Zaipobly, whereas no stationary health supply is provided in other villages (e.g. Pauleoula) (observation from 2012). The only physician (supported by international physicians of the "Médecins sans frontières") works in the hospital in Taï, where basic medical treatments are provided and basic operations performed. In 2012, a diagnostic laboratory was in construction, but the financial support for the equipment acquisition was still uncertain. Except for Keibly, where the well-furnished "case de santé" is managed by a nurse, health care is mainly provided by the "Agents the santé" (contact person for health care) or local "marabouts" (local sorcerer providing traditional medicine).The next larger general hospital is located in Guiglo. In 2012, many different NGO`s (among others "Médecins sans frontières", "Croix rouge" and "Danish Refugee Council") worked in the Taï region in close collaboration with the local hospital and the "Agents de santé". Moreover a camp of the ONUCI (United Nations Operation in Côte d`Ivoire) was based between Taï village and Daobly.

Figure 2.Picture of life in Taï village and the proximate tropical rain forest

3.2 Sample and data collection

An international and interdisciplinary team (including among others German and Ivorian biologists, an Ivorian physician and myself) collected the human and animal samples during two field missions in in April-June and September-October 2012 in 8 villages in the Taï region.

Collection of human samples. In total 648 samples from volunteering participants were collected in 8 villages, scattered on the logging roads along the Taï National Park: 115 in Ponan, 95 in Daobly, 30 in Taï, 96 in Gouleako, 85 in Pauleoula, 67 in Zaipobly, 95 in Gahably and 65 in Keibly. Although many different samples (nasal, oral and skin swabs, blood, urine and feces) were collected from nearly every participant, only the fecal samples were tested in the current study. All participants were instructed on the proper method for fecal collection to avoid contamination. Plasma, buffy-coat and erythrocytes were obtained 4 to 8 hours after sampling and kept frozen in liquid nitrogen. Swabs, urine and fecal samples were also conserved in liquid nitrogen and in RNAlater medium. All the samples were transferred to a -80°C freezer in Côte d`Ivoire and transported to Germany on dry-ice *(Figure 3)*.

A basic clinical examination was performed by a trained medical professional and, when necessary, free treatment and medical advice were provided. If treatment was not possible on site, individuals were referred to an appropriate medical facility. Besides free treatment, every participant obtained a bar of soap and a package of biscuits. The final written medical history included socio-demographic data including among others age, sex, location of residence, birthplace, ethnicity and information about current medical condition. Participants were assigned to one of three age groups: young children (0-5 years old), older children and adolescents (6-19 years old), and adults (20 years and older). Although everyone was invited to participate to the study, more women than men volunteered (402 compared to 238, gender data missing for 8 participants) (*Table 4 and Table 5*). The mean age of the participants was 33 years (range 0-80 years) and the most frequently reported occupation for men was agriculture (mainly of cocoa and rubber) and for women housewife.

A randomly chosen subset of this sample collection was tested with different PCR systems. This subset comprised in total 189 fecal samples. Only 95 samples from this subset were included in the dataset for the paper on prevalence of HAdV D in sub-saharan countries (Pauly *et al.* 2014). Moreover, fecal samples collected with a similar approach in other African countries were tested fro HAdV-D. The volunteers from DRC (Salonga National Park) (n=105), were predominantly hunters, livestock breeders and cultivators without regular contact to other populations and were recruited from 4 villages. Participants were between 0 and 78 years old (mean= 28). Stool was also sampled from adult male field assistants of Dzanga-Sangha Protected Areas (Central African Republic, n=18) and Bwindi National Park (Uganda, n=69). For these 2 study groups (from Central African Republic and Uganda), clinical data were not recorded.

Before sampling, the aim of the study, as well as the possibility to quit the study at any point was explained individually in the local language. An individual study number was assigned to every participant in order to protect the privacy of the participant. Written informed consent was obtained from every study participant before sampling and the collection was approved by the responsible ethic commission of every country and was performed according to the declaration of Helsinki.

Questionnaires. A standardized questionnaire was designed to provide basic demographic information, as well as personal epidemiological data. The questionnaire focused particularly on exposure to domestic animals and wildlife via farming, pets or bushmeat-related activities (e.g. hunting, dismembering, cooking and eating bushmeat). The questionnaire also aimed at identifying all occupational exposures to animal tissues and body fluids during hunting or meat preparation. Moreover, we wanted to elucidate whether the domestic animals have already been treated by a veterinarian and which animal products the local population consume and use. The local interviewers were well-trained and explained the study objectives and procedures prior to interviewing. The questions were consistently filled in by a team member. Although the questionnaire was in French, interviews were also conducted in local languages (e.g. Dioula). The questionnaire data were treated anonymously and only the study number was used to relate the samples and the questionnaires of each study participant *(Figure 3, Supplementary Figure 2 and Supplementary Figure 3).*

N° samples/Village	Gender	Birth country	Age at sampling	Ethnicity	Occupation
Ponan: 115	F: 402	Côte d`Ivoire: 382	Min.: 0 years	Mossi: 268	Housewife: 290
Gouleako: 96	M: 238	Burkina Faso: 195	Median: 30 years	Guere: 115	Grower: 151
Gahably: 95	n.a.: 8	Mali: 24	Mean: 33 years	Ubi: 75	Pupil/Student: 57
Daobly: 95		Benin: 1	Max.: 80 years	Baoule: 49	Seller: 27
Pauleoula: 85		Ghana: 1	n.a.: 25	Grussi: 28	Farmer: 11
Zaipobly: 67		Guinée: 1		Other: 97	Other : 47
Keibly: 65		n.a.: 44		n.a.: 16	n.a.: 65
Taï: 30					

Table 4.Descriptive statistics of demographic data of the human study participants from CI

For some participants the demographic data were incomplete

	Ponan	Taï	Daobly	Gouleako	Keibly	Pauleoula	Gahably	Zaipobly
Female	83	27	50	51	20	61	60	50
Male	32	3	44	45	42	24	34	14
Major ethnical group	Mossi	Baoulé	Mossi	Ubi	Guéré	Ubi	Mossi	Mossi

Table 5.Descriptive statistics of demographic data of the study participants in each village

For some participants the demographic data were incomplete.

Figure 3. Pictures of the team collecting samples of the human volunteers (left) and doing a questionnaire (right)

Collection of animal samples. Besides collecting human samples during the 2 field missions in Côte d`Ivoire in 2012, 554 samples were collected from different domestic animal species. Similarly as for the human samples, all animal owners were invited to participate in the study. In exchange, the sampling team offered basic medical treatment if needed and provided anti-parasitical medication for the animals. A randomly chosen subset of this sample collection was tested with different PCR systems. This subset comprised in total samples from 321 animals: 14 cows, 58 dogs, 60 goat, 7 monkeys, 24 pigs, 50 sheep and 91 chickens (*Figure 4)*. Moreover 17 rats were caught in the villages, mainly inside the human habitations, and tissue samples were obtained during full necropsies, carried out under extensive safety precautions. The samples were obtained from 8 villages: Daobly (n=60), Gahably (n=32), Gouleako (n=55), Pauleoula (n=41), Keibly (n=32), Ponan (n=41) and Taï (n=26). Furthermore data on sex (male/female), age (adult/juvenile), body temperature (°C), nutritional and coat condition (good/moderate) were obtained from most of the sampled animals (excluding the chickens).

Although many different samples (nasal, oral, rectum/cloacal and skin swabs and blood) were collected from nearly every animal, only the rectum swabs were tested in the current study. From the samples, collected from rodents, only the intestine and spleen samples were tested. Plasma, buffy-coat and erythrocytes were obtained 4 to 8 hours after sampling and kept frozen in liquid nitrogen. Swabs and fecal samples were also conserved in liquid nitrogen and in RNAlater medium. All samples were transferred to a -80°C freezer in Côte d`Ivoire and transported to Germany on dry-ice.

The sampling missions were approved in November 2010 by the ethic commission "Comité national d'éthique et de la recherche (CNER)" from the "Ministère de la santé et de l'hygiène publique – République de Côte d`Ivoire". The permit number is 101-10/MSHP/CENR/P.

Figure 4. Pictures of the team collecting samples of domestic animals

3.3 Consumables

Consumables for sampling

During the field missions a huge amount of material was necessary for the sampling, but also for the processing of the samples in the field laboratory. The list below is non-exhaustive and comprehends only the key sampling materials.

Cryotubes 2.0ml	Roth
Cotton swabs sterile Plastic	Heinz Herenz
Monovette	Sarstedt
Feces tubes with spoon	Sarstedt
Needles for Monovette 20 G, 22 G	Sarstedt
Sterilium	Hartmann
Gloves M nitril	Rotiprotect
Disposable Aprons	Roth
Nitrogen container Cryo Diffusion B2036	Bayern Genetik GmbH

Buffers and solutions

100 bp DNA Ladder	New England BioLabs® Inc.
10x ExTaq buffer	TaKaRa Bio Inc.
dNTP Mix 2,5 mM each	TaKaRa Bio Inc.
dNTP Mix 4x 10 mM in water	Metabion international AG
GelRed Nucleic Acid STaïn 10,000x in DMSO	Biotium Inc.
Loading buffer: 5 parts Bromphenolblue-solution + 7 parts 70 % Sucrose-solution	FG 12, Robert Koch-Institut
$MgCl_2$-solution 25 mM	Applied Biosystems®
PCR buffer II (10x) contains no $MgCl_2$	Applied Biosystems®
Quick load 1 kb Extend DNA Ladder 50 ng/µl	New England BioLabs® Inc.
UltraPure™ 10x TBE (1 M Tris, 0,9 M boric acid, 0,01 M EDTA)	Invitrogen™ life technologies
Water for molecular biology (PCR-grade water), nuclease free	Sigma-Aldrich Co. LLC

Chemicals

Agarose (elekrtophoresis grade)	Invitrogen™ life technologies
Bromphenol blue	SERVA Electrophoresis GmbH
Dimethylsulfoxide (DMSO)	Sigma-Aldrich
Ethanol rotipuran ≥ 99,8 %	Carl Roth GmbH + CO. KG
Sucrose	Sigma-Aldrich Co. LLC

Oligonucleotides for PCR and sequencing

All primers were synthesized by Metabion International AG, Martinsried, Germany. The only exceptions were the blocking primers, which were synthesized by TIB Molbiol GmbH, Berlin; Germany. For more information about the applied primers and their nucleotide sequence *(Table 8)*.

Enzymes

AmpliTaq Gold® 5 U/µl	Applied Biosystems®
TaKaRa Ex Taq® 5 U/µl	TaKaRa BIO Inc.

Kits

BigDye® Terminator v3.1 Cycle Sequencing Kit	Applied Biosystems®
GeneMATRIX Stool DNA Purification Kit	Roboklon GmbH
Invisorb® Spin DNA Extraction Kit	Stratec molecular GmbH
NucleoSpin® RNA II	Macherey-Nagel GmbH & Co. KG

3.4 Equipment

Centrifuge 5417R	Eppendorf
Centrifuge Heraeus Fresco 17	Thermo Schientific
FastPrep®-24	MP Biomedicals
Geldocumentation BioDocAnalyze	Biometra GmbH
NanoDrop ND-1000 spectrophotometer	peqlab
Sequencer: 3500xL Dx Genetic Analyzer	Applied Biosystems®
Standard Power Pack P25	Biometra GmbH
T Professional Thermocycler	Biometra GmbH
Thermo shaker TS-100	Kisker Biotech GmbH & Co. KG
ThermoStat plus	Eppendorf

3.5 Software

BioDocAnalyze 2.2	Biometra GmbH
FigTree Drawing Tool v1.4.0	(Rambaut and Drummond 2014)
Gblocks 0.91b	(Castresana 2000, Talavera and Castresana 2007)
Geneious 7.1.4	(Kearse *et al.* 2012)
jModelTest v2.1.4	(Darriba *et al.* 2012)
Lasergene® SeqMan Pro™	DNASTAR, Inc.
NanoDrop ND-1000 v.3.7.1	Thermo Scientific
PhyML 3.0 online	(Guindon and Gascuel 2003, Guindon *et al.* 2005)
Seaview v4	(Gouy *et al.* 2010)
Prottest v3	(Abascal *et al.* 2005, Darriba *et al.* 2011)
R Software i386 3.0.2.	(R-Core-Team 2014)
BEAST v1.8.0	(Drummond *et al.* 2012, Bouckaert *et al.* 2014)
Tracer v1.6	(Rambaut A and Drummond 2009)
Fabox v1.41	(Villesen 2007)
Primer 3 plus	(Untergasser *et al.* 2007)
Rdp 4	(Martin *et al.* 2005, Martin *et al.* 2010)
PAUP	(Swofford 2003, Wilgenbusch and Swofford 2003)

3.6 Laboratory techniques

The laboratory analyses were performed at the Laboratoire National d'Appui au Dévelopment Agricole (LANADA) in Abidjan, Côte d`Ivoire, and at the Robert Koch-Institute (RKI) in Berlin, Germany. Randomly, some of the PCR experiments carried out in Côte d`Ivoire were repeated in Germany to control for accuracy of the results. The methodological approach applied is summarized on Figure 5.

Strict measures were followed to avoid contamination with genomic DNA and PCR products and false-positive results. Already during the field mission, collection and processing of human and animal samples were physically separated. Gloves were worn during sample collection and the samples were placed in sterile tubes. The samples were not only collected by different sampling teams, but were even processed in different rooms of the field laboratory and stored in different liquid nitrogen containers. In the laboratory in Côte d`Ivoire only the human samples were analyzed and strict laboratory rules established. The fact that research of the African partners focuses mainly on RNA-viruses further limited the contamination risk. Upon arrival in Germany, the human and animal samples were separated in different cryoboxes. Moreover, DNA extraction and the PCRs with the human and animal samples were performed in different laboratories of the RKI. In every PCR run and extraction set, a negative control was included. For the PCRs, positive controls were chosen to fulfill following criteria: 1) detectable by the particular PCR and 2) different from the AdV strain expected in tested sample (e.g. bat AdV as positive control while testing domestic animals).

It was considered that fecal samples and rectum swabs would be a well-adapted material for the study purpose as long-lasting shedding of AdV in feces has been described for humans, but also for many animal species and as shedding of AdV has been often associated with disease (McFerran *et al.* 2000, Horwitz *et al.* 2007, Roy *et al.* 2009).

Applied AdV Taxonomy. The partly ambiguous taxonomy of AdV might give rise to confusion while reading this thesis. The standard taxonomic approach is to name a novel AdV species after the host in which the AdV was first detected. This is problematic as some AdV species have a broad host range (e.g. HAdV and bovine AdV). Hence, HAdV are frequently detected in NHP and bovine AdV in sheep (Lehmkuhl *et al.* 1993, Wevers *et al.* 2011). In this thesis the official AdV taxonomy was applied (Harrach *et al.* 2011) and the animal host species was always precised in order to avoid misunderstandings (e.g. HAdV was detected in rectum swabs collected from dogs).

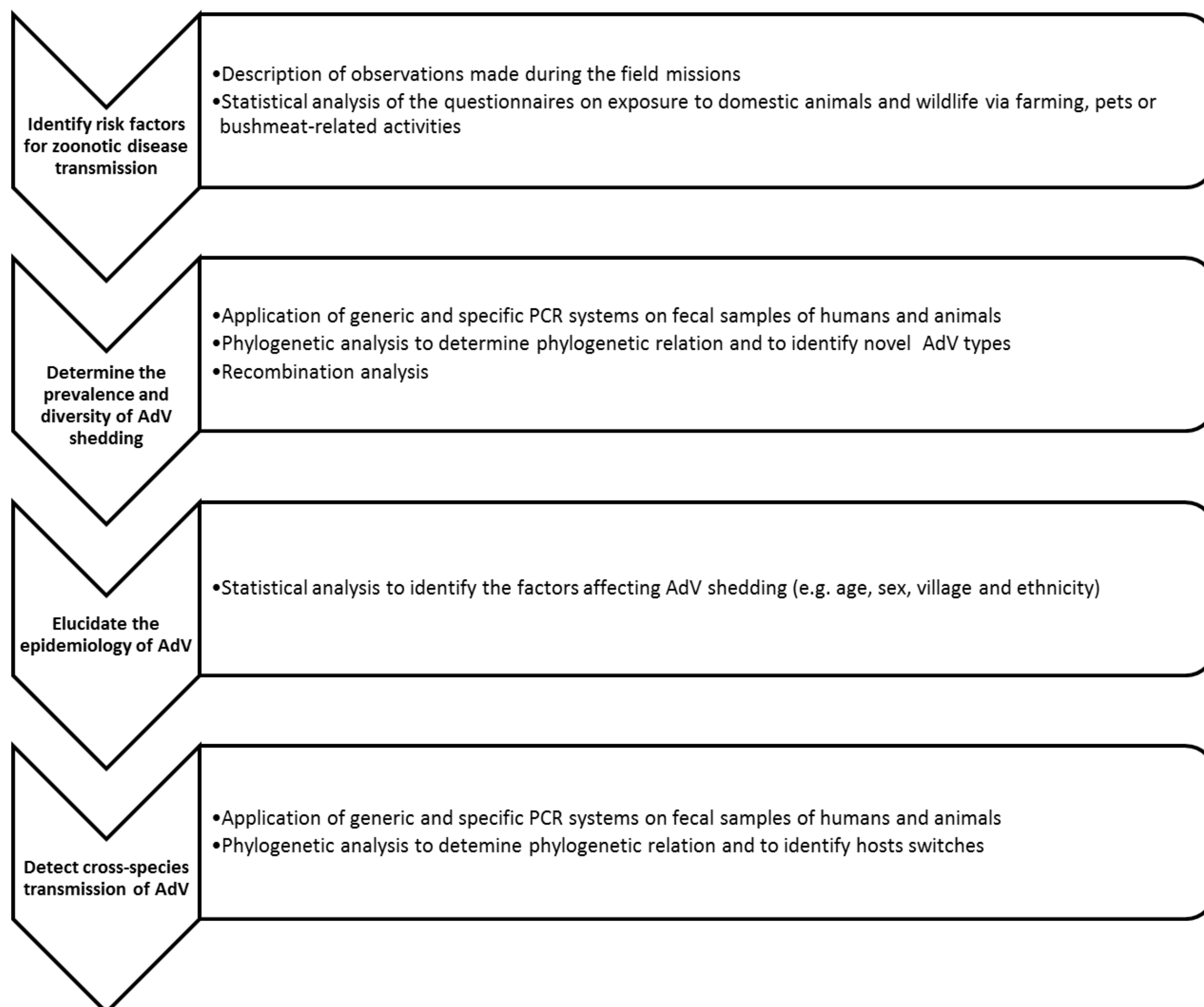


Figure 5. Schema of the methodical approach

DNA extraction

DNA-extraction was performed using the Qiagen-DNeasy Blood & Tissue Kit for the rectum swabs and the Roboklon EURx GeneMatrix Stool DNA purification Kit for the feces samples, according to the manufacturer`s instructions. Regarding the cell lysis step, the manufacturer`s protocol for the feces extraction was modified, as no BeadBeater was available in the laboratory in CI. Instead, the bead tubes, containing the sample, the buffer and the carrier-RNA, were incubated during 4-5 hours at room temperature and vortexed every 30 min. The extracted DNA was stored at -20 °C. To enable also research on RNA viruses, 45 µl of the eluate were aliquoted and stored at -80 °C. An aliquot of 5 µl was taken for measurement of the DNA concentration with NanoDrop ND-1000 spectrophotometer at 280 nm.

PCR

Many of the diagnostic PCR systems applied in the current study for the detection of AdV were newly designed in order to answer the specific research question of the study; others had been established before and were adopted without or only with slight modifications. The target of most PCR experiment was the complete hexon gene or a fragment of it. In addition, two PCR systems were applied that target the viral DNA polymerase gene, which has emerged in a number of studies as an optimal target for generic PCR of AdV and other larger DNA viruses.

Most of the PCR systems were designed in a nested or semi-nested format. Nested PCR means that 2 pairs of PCR primers are applied in 2 subsequent rounds of PCR, whereby the second pair binds within the locus amplified by the first pair; in other words, the target of the second pair is nested within the target of the first pair. After a first complete PCR cycle with the first pair, the second pair is applied to the PCR product obtained during the first amplification. The aim of a nested PCR is to increase specificity of the PCR by decreasing the probability of non-specific amplification, due to the amplification of unintended primer binding sites (mispriming). Moreover, the detection limit of the PCR is lowered, which is advantageous if low amount of target sequence is expected in the sample. A draw-back of the method is the potentially increased rate of false positives due to contamination during the manipulation between the two PCR runs. In fact, before the second primer pair can be applied, the tubes containing high concentrations of the first amplification product must be opened and manipulated to set up the second amplification round.

For all conventional PCR systems, the same PCR mix was used. It contained 1x AmpliTaq™ buffer, 2.0 mM $MgCl_2$, 2-5 % DMSO, dNTPs at 200 mM each, 2.0 units of AmpliTaq Gold Polymerase and 1 µM of each primer pair. Thermocyclers of type TgradientS (Biometra, Germany) were utilized. The PCR program involved an initial denaturing at 95 °C for 12 min, followed by 45 cycles of denaturing at 95°C for 30 s, annealing at the primer-specific temperature for 30 s, and elongation at 68°C for 2-3 min. The final elongation was performed at 72 °C for 15-30 min. In the second round of the nested PCRs, 1-1.5 µl of reaction mixture from the first round was used as target with an identical cycling program.

Primer design was performed manually and based on an alignment of the target sequences. Before ordering, primers were tested for self-complementarity with the online Software primer3plus (Untergasser *et al.* 2007). The software would also calculate primer length, base composition and melting temperature. Some of the generic PCR systems applied included degenerate primers. A PCR primer sequence is called degenerate if some of its positions, called “wobbles”, have several possible bases. This means that during one PCR run not a single specific pair of primers is applied, but that PCR primers with related sequences that present different nucleotides at specific locations are applied simultaneously, i.e., as a primer mix. Degenerate primers are applied if the target sequence is not exactly known or if similar sequences of a variety of related species is to be amplified. By including inosine residues in the primer sequence, the target range can be further increased, as inosine residues can pair with any nucleotide.

PCR and cycling conditions *(Table 6)* were optimized by testing all primers at different annealing temperatures and with different dilutions of purified virus as positive control in order to determine the cycle conditions, permitting specific primer annealing and avoiding mispriming. *A similar*

*approach was applied when screening the human and animal study population for AdV. In a first step, all samples were tested with different generic PCR systems to sensitively determine AdV prevalence and to identify the circulating AdV species. In a second step, PCR systems targeting a longer genome fragment of the detected AdV were applied to identify the AdV type and to perform phylogenetic and recombination analysis. In a last step, specific primers were applied on a subset of samples depending on the research question (e.g. detection of siadenoviruses, of HAdV-B or of zoonotic transmission of AdV) (*Table 7*).*

N° of cycles	Temperature	Time duration	Step
1 x	95 °C	12 min	Initialization
	95 °C	30 s	Denaturation
45 x	T_m* °C	30 s	Primer annealing
	68 °C	2-3 min	Elongation
1 x	72 °C	15-30 min	Final elongation
1x	16°C	∞	Final hold

Table 6. PCR cycling condition

T_m: Annealing Temperature*

	PCR system applied to			
Aim	**human samples**	**domestic mammalian samples**	**chicken samples**	**rodent samples**
1) First screening for AdV	DPOL PCR	DPOL PCR	short HEX-FAdV PCR	Animal-HEX PCR
		Animal-HEX PCR	DPOL PanAdV PCR	
		DPOL PanAdV PCR		
2) Characterization of detected AdV	LD-HEX-HAdVD PCR	LD-HEX-MastAdV PCR	long HEX-FAdC PCR	LD-HEX-MastAdV PCR
3) Specific detection of a certain AdV species	HEX-HAdVD PCR	n.d.	SiAdV-HEX-PCR	n.d.
	ruminant-HEX PCR			
	Animal-HEX-PCR+Blocking-HAdVD			

Table 7. Methodological approach for AdV screening of human and animal samples with PCR

n.d: not done

The details on annealing temperature, primer sequence, target gene, etc. of a particular PCR system can be retrieved from the Table 8 and in the following sections.

➔ Testing of the human stool samples

All human stool samples were initially analyzed for the general presence of AdV, primarily with a generic nested PCR system that targets the DNA polymerase gene (Wevers *et al.* 2011) and then with genus-specific nested PCR systems, that target the hexon gene. In case of HAdV-D characterization, longer fragments, comprising the genes pVII-hexon, were obtained with Long-Distance PCR.

Generic HAdV PCR (DPOL-PCR). The DPOL PCR applied here, is generic for most mastadenoviruses and detects mainly primate (simian and human), but also several other mammalian AdV. These nested degenerate and deoxyinosine-substituted primer pairs have been described before and were initially designed to detect human and simian AdV (Wellehan *et al.* 2004, Wevers *et al.* 2011). The PCR amplifies a fragment of approx. 955bp in the first round, and of approx. 613bp in the second round of PCR. The target sequence lies within the DNA polymerase gene (DPOL) gene *(for the exact position and sequence see Table 8)*.The first round of the nested PCR was performed with 5 µl of DNA extracted from human stool as template, 12.7 µl of PCR mix and 1 µM of each first round sense and antisense primer and PCR-grade H_2O ad 25 µl. In second round amplification, 1.5 µl of the first-round amplification product were added to 12.7 µl of PCR mix with 1 µM of each second round primers and H_2O ad 25 µl. The following cycle conditions were applied during the two amplification rounds:

activation of the polymerase at 95ºC for 12 min and 45 cycles of denaturation (95°C, 30 s), annealing (45°C, 30 s), and elongation (72°C, 2 min), final elongation at 72°C for 10 min.

Specific nested HAdV D PCR (HEX-HAdVD PCR). For the generic detection of members of the species HAdV D, non-degenerate primers were designed, based on an alignment of published HAdV D sequences. The used nested degenerate and deoxyinosine-substituted primers have been described before and were initially designed to detect HAdV D in humans and non-human. These primers amplify a fragment of approx. 314bp in the first round, and of approx. 281bp in the second round of PCR. The target sequence lies at the end of the hexon gene *(for the exact position and sequence see Table 8)*. The nested PCR was performed as described for the DPOL PCR under the following cycling conditions: activation of the polymerase at 95ºC for 12 min and 45 cycles of denaturation (95°C, 30 s), annealing (63°C, 30 s), and elongation (72°C, 2 min), final elongation at 72°C for 10 min.

Specific Long-Distance-PCR (LD-HEX-HAdVD PCR). Long-Distance nested PCR was performed using the TaKaRa-EX PCR system according to the instructions of the manufacturer (Takara Bio Inc., Otsu, Japan) and resulted in a fragment of approx. 4.8 kb. This nested PCR included 3 rounds of amplification. These primers amplify a fragment of approx. 5.4 kb in the first round, of approx. 4.9 kb in the second round, and of approx. 4.7 kb in the third round of PCR (5396 bp, 4911 bp and 4724 bp in AdV xy). The sense primers bind at the 3´-end of the pVII gene and the antisense primers at the 3´-end of the hexon gene *(for the exact position and sequence see Table 8)*. Depending on the DNA concentration, up to 7 µl of DNA, were added in the first round to 15.5 µl of PCR mix (containing 10× ExTaq buffer with MgCl2, dNTPs at 2.5 mM each, 5.0 units of Ex Taq Polymerase and 10 µM of each first round sense and antisense primer) and PCR-grade H_2O ad 50 µl. In second and third round amplification, 1 µl of the reaction product generated in the preceding PCR round, was added to 15.5 µl of PCR mix with 10 µM of the respective primers and H_2O ad 50 µl. Thermocyclers of type TgradientS (Biometra, Germany) were used under the following conditions for every round: activation of the polymerase at 94ºC for 5 min and 15 cycles of, followed by 15 cycles of denaturation (98°C, 20 s), annealing (60°C, 30 s), and elongation (68°C, 8min + 5 s) and a final elongation at 72°C for 30 min.

➔ Testing of the animal rectum/cloacal swab samples

Generic AdV PCR of mammalian samples

To estimate the diversity of AdV shed by the sampled animals, different primer pairs were applied that amplify orthologous (same gene and same genomic position) DNA regions of the genus *Mastadenovirus*.

All mammalian samples were initially tested for the general presence of mastadenoviruses, first with the generic DPOL-PCR described above (Wevers *et al.* 2011) and second with a generic semi-nested PCR that targets the hexon gene of all mastadenoviruses. Then, longer fragments of the detected AdV were amplified with Long-Distance PCR. In order to investigate the occurrence and prevalence of AdV from other genera, all ruminant samples were further tested with a generic PanAdV PCR that

targets the DNA polymerase gene of AdV from presumably all genera (see "Generic FAdV PCR of chickens' samples") (Wellehan *et al.* 2004).

DPOL PCR. *See 3.6 Laboratory techniques "Testing of human feces samples".*

Generic semi-nested MastAdV PCR (Animal-HEX PCR). For the generic detection of all mastadenoviruses, degenerate primers were designed based on an alignment of published animal AdV (ruminant, canine, equine, bat and murine), simian and human AdV sequences. These primers amplify a fragment of approx. 292 bp in the first round, and of approx. 160 bp in the second round of PCR. The target sequence lies at the beginning of the hexon gene *(for the exact position and sequence see Table 8)*. The first round of the semi-nested PCR was performed with 12.3 µl of DNA extracted from animal rectum swabs as template, 12.7 µl of PCR mix and 1 µM of each first round sense and antisense primer and PCR-grade H_2O ad 25 µl. In order to increase the specificity and to reduce mispriming of the PCR primers, only 1 µl of a 40-fold diluted first round PCR product was used in the second round PCR. This 1 µl was added to 12.7 µl of PCR mix with 1 µM of each second round primers and H_2O ad 25 µl. Thermocyclers of type TgradientS (Biometra, Germany) were used under the following conditions: activation of the polymerase at 95°C for 12 min and 45 cycles of denaturation (95°C, 30 s), annealing (56°C, 30 s), and elongation (72°C, 2 min), final elongation at 72°C for 10 min.

Generic Long-Distance Mastadenovirus PCR (LD-HEX-MastAdV PCR). Long-Distance nested PCR was performed using the TaKaRa-EX PCR system according to the instructions of the manufacturer (Takara Bio Inc., Otsu, Japan) and resulted in a fragment of about 2.7kb. The sense primers bind to the 5´-end and the antisense primers to the 3´-end of the hexon gene *(for the exact position and sequence see Table 8)*. The first round primers have been described before (Lehmkuhl *et al.* 2008) and the second round primers were designed based on an alignment of published animal (ruminant, canine, equine, bat and murine) AdV sequences. LD-HEX-MastAdV PCR was performed as described for the LD-HEX-HAdVD PCR under the following cycling conditions for each round: activation of the polymerase at 94°C for 5 min and 15 cycles of, followed by 15 cycles of denaturation (98°C, 20 s), annealing (first round: 45°C, 30 s; second round: 55°C, 30 s), and elongation (68°C, 8 min + 5 s) and a final elongation at 72°C for 30 min.

Generic FAdV PCR of chickens' samples

All chickens' samples were tested for FAdV, primarily with a generic nested PCR that targets a short fragment of the hexon gene. Then, the positive samples were retested with primers amplifying a longer fragment of the hexon gene of all published FAdV (Meulemans *et al.* 2001). With these longer fragments, phylogenetic analyses with good branch support and delineation analyses of the detected FAdV were feasible. In order to investigate the occurrence and prevalence of avian AdV from other genera, all chickens samples were additionally tested with a generic PCR that targets the DNA polymerase gene of AdV from presumably all AdV genera (Wellehan *et al.* 2004).

Generic nested Fowl AdV PCR (short HEX-FAdV PCR). For the generic detection of FAdV (genus *Aviadenovirus*), degenerate primers were designed based on an alignment containing at least one

published representative of all 12 known FAdV types. These primers amplify a fragment of approx. 390 bp in the first round, and of approx. 306 bp in the second round of PCR. The target sequence lies at the beginning of the hexon gene *(for the exact position and sequence see Table 8)*. The nested PCR was performed as described for the Animal-HEX PCR (without the dilution step after the first round) under the following cycling conditions: activation of the polymerase at 95°C for 12 min and 45 cycles of denaturation (95°C, 30 s), annealing (45°C, 30 s), and elongation (72°C, 2 min), final elongation at 72°C for 10 min.

Generic nested Fowl AdV PCR (long HEX-FAdV PCR). To obtain a longer fragment of the FAdV detected with the primer of the short HEX-FAdV PCR, we retested the positive samples with primers described before and which were initially designed to detect all FAdV types (Meulemans *et al.* 2001). These primers amplify a fragment of approx. 862 bp in the first round, and of approx. 547 bp in the second round of PCR. The target sequence lies at the beginning of the hexon gene *(for the exact position and sequence see Table 8)*. The nested PCR was performed as described for Animal-HEX PCR (without the dilution step after the first round) under the following conditions: activation of the polymerase at 95°C for 12 min and 45 cycles of denaturation (95°C, 30 s), annealing (54°C, 30 s), and elongation (72°C, 2 min), final elongation at 72°C for 10 min.

Generic nested PanAdV PCR of ruminant and chicken samples (DPOL-PanAdV PCR). To gain more knowledge about the AdV diversity in respect to AdV types and genera circulating in the study area, all samples from ruminants (cows, sheep and goat) and chickens were tested with generic primers targeting the polymerase gene of presumably all known AdV genera. This PCR system has been used in several studies to discern the biodiversity of AdV in different animal species (Wellehan *et al.* 2004, Rivera *et al.* 2009, Wellehan *et al.* 2009, Kovacs *et al.* 2010, Janoska *et al.* 2011, Kajan *et al.* 2011). Although the PCR system was initially designed to detect AdV in lizards, it successfully amplified at- and mastadenoviruses (Wellehan *et al.* 2004). These primers amplify a fragment of approx. 508 bp in the first round, and of approx. 275 bp in the second round of PCR. The target sequence lays at the beginning of the polymerase gene *(for the exact position and sequence see Table 8)*. The nested PCR was performed as described for the Animal-HEXON-PCR (without the dilution step after the first round) under the following cycling conditions: activation of the polymerase at 95°C for 12 min and 45 cycles of denaturation (95°C, 30 s), annealing (46°C, 30 s), and elongation (72°C, 2 min), final elongation at 72°C for 10 min.

Specific SiAdV PCR (SiAdV-HEX-PCR). To confirm the presence of siadenoviruses and to obtain longer fragments of the siadenovirus types detected with the DPOL-PanAdV PCR, the positive samples were retested with degenerate primers, which had been designed based on an alignment containing all the published siadenovirus types. These primers amplify a fragment of approx. 538 bp. The target sequence lays at the beginning of the hexon gene *(for the exact position and sequence see Table 8).* The PCR was performed as described for the Animal-HEX PCR (without the dilution step after the first round) under the following cycling conditions: activation of the polymerase at 95°C for 12 min and 45 cycles of denaturation (95°C, 30 s), annealing (54°C, 30 s), and elongation (72°C, 2 min), final elongation at 72°C for 10 min.

➔ Testing for zoonotic transmission of AdV

The aim of this part of the project was to ascertain whether zoonotic transmission of AdV occurs. We assumed that shedding of "animal" AdV by humans is a first indication for zoonotic transmission of AdV or at least for a recent spill-over. Hence all human fecal samples were tested for animal AdV. Different approaches were used to assure amplification of even small amounts of animal virus and to simultaneously limit the amplification of the closely related and highly prevalent human AdV.

In fact a major problem to face, when PCR is applied to a human sample that contains genome sequences of multiple AdV, is that the predominant target sequence is preferentially amplified. Here, it was supposed that the human AdV that are highly prevalent in human samples would prohibit sensitive detection of animal AdV (the AdV of interest), resulting in false-negative results because of template competition during the PCR. Several approaches have been applied to deal with this obstacle.

A first approach to tackle the problem is to design primers that amplify DNA regions, where the AdV of interest and non-target sequence have diverged, i.e., that discriminate between human and animal AdV. This approach is not promissing when the target of human and animal AdV are too similar or when more than 1 animal AdV is to be amplified from a sample. This was the case in the present study, as the aim was to detect any animal AdV in human stool samples. Moreover, most animal and all human AdV belong to the genus *Mastadenovirus*. and thus display a considerable degree of similarity thereby frequently preventing the design of discriminating primers, i.e., it was impossible to design primers, which target a DNA region highly conserved among all the animal AdV and distinct from human AdV. Another approach is to design multiple primer pairs, each amplifying one animal AdV sequence and excluding human AdV sequences. A disadvantage of this method is that unanticipated targets are overlooked. Moreover, different PCR enrichment strategies, such as combined amplification and restriction digestion, DNA blockers and peptide nucleic acid (PNA) clamps, have been described to approach the problem. Blocking primers have been used in different studies. For example to identify the diet of predators or of the Antarctic krill or to investigate the mammal diversity in ancient permafrost samples from Siberia (Vestheim and Jarman 2008, O'Rorke *et al.* 2012). Blocking primers are DNA oligonucleotides, which preferentially bind to the contaminant DNA and whose 3` end is modified, resulting in inhibition of enzymatic elongation of the primer. There are two common methods for the design of blocking primer reactions. In the first method, 'primer exclusion', blocking primers impede elongation by binding the sequenceof non-interest in between the two amplification primers. In the second, 'elongation arrest', the primers compete directly with the amplification primers (Vestheim *et al.* 2008). By adding blocking primers against the most prevalent human AdV (HAdV-D) to a generic PCR system amplifying all mastadenoviruses, we obtained a specific and sensitive PCR system amplifying exclusively the animal AdV. In the present study a C3 spacer was added at the 3`end of the blocking primer sequence. The objective was that these blocking primers would prevent the amplification of HAdV D sequences, but would allow the amplification of animal AdV sequences.

Two approaches for the investigation of zoonotic transmission of AdV were applied in the current study. In a first approach, the blocking primers were applied together with the generic Animal-HEXON-PCR primers established before (see above). This allowed the selective amplification of

animal AdV. In a second approach all human samples were tested with primers specifically targeting FAdV and ruminant AdV.

Animal-HEX PCR with blocking primers against HAdV D (Blocking-HAdVD PCR). To permit the detection of animal mastadenoviruses in humans, we designed blocking primers against HAdV D, the HAdV species most frequently shed by humans in sub-Saharan Africa (Pauly *et al.* 2014). For every step of the semi-nested Animal-HEX PCR, a degenerate sense 5´-3´ blocking primer was designed based on an alignment of the hexon genes from a selection of animal AdV and the 14 HAdV D 2.7kb long sequences, which were amplfied with the LD-HEX-HAdVD PCR from humans living in the investigated area (see above) *(for the exact primer position and sequence see Table 8).*

Of the two common approaches to design blocking primer reactions, 'primer exclusion' and 'elongation arrest', the `primer exclusion` approach was chosen as it has been proven to be more efficient (Vestheim *et al.* 2008). Other studies revealed the high efficiency of annealing blocking primers which partially overlap with the amplification primer (Vestheim *et al.* 2008). Thus the applied blocking primers partially overlapped with the primer-binding site of the Animal-HEX PCR, were complementary to HAdV D hexon sequences and non-complementary to those of the other mastadenoviruses. The inhibition is due to the ligation of a C3-Spacer *CPG* (1-dimethoxytrityloxy-propanediol-3-succinoyl-long chain alkylamino-CPG) extension at the 3´-end. It is this modification thatprohibits the polymerization in 3`-direction on HAdV D templates.It is important to keep the blocking primer concentration at a minimum in order to avoid unintended blocking of the target sequence. Thus, in exploratory experiments before the screening, different concentrations of blocking primers were tested on different concentrations of target and contaminant DNA to elucidate the amount of blocking primers needed.

The specificity of the blocking primers was tested on animal samples that had already been tested positive for animal AdV with the Animal-HEX-PCR. The sensitivity of the primers was tested at different annealing temperatures on HAdV D-positive human fecal samples. Moreover, it was investigated if adding blocking primers only in one step of the semi-nested PCR would be sufficient. All tests were run successfully and the PCR protocol optimized to ensure a specific and sensitive amplification, accurately revealing individuals infected with was animal AdV. Finally, a 5-fold excess of the blocking primers compared to the hexon-primer (ratio 5:1) seemed sufficient for blocking nontarget DNA and was applied in every PCR-reaction. Blocking primers were added at each step of the semi-nested PCR and the seminested PCR was performed as described for the Animal-HEX PCR under the following conditions: activation of the polymerase at 95°C for 12 min and 45 cycles of denaturation (95°C, 30 s), annealing (56°C, 30 s), and elongation (72°C, 2 min), final elongation at 72°C for 10 min.

short HEX-FAdV PCR. After ascertaining that this PCR system specifically amplificated exclusively FAdV and not HAdV, the human stool samples were tested for the presence of FAdV. For PCR conditions, *see short HEX-FAdV PCR*.

Specific nested ruminant AdV PCR (ruminant-HEX PCR). To further investigate the zoonotic potential of AdV, primers targeting exclusively the hexon gene of ruminant AdV (ovine, caprine and bovine) and not human AdV, were designed, based on an alignment of published animal (ovine, caprine and bovine) and human AdV sequences. These primers amplify a fragment of approx. 394 bp in the first

round, and of approx. 286 bp in the second round of PCR. The target sequence lies within the hexon gene (*for the exact position and sequence see Table 8)*. The nested PCR was performed as described for the DPOL PCR under the following conditions: activation of the polymerase at 95°C for 12 min and 45 cycles of denaturation (95°C, 30 s), annealing (61°C, 30 s), and elongation (72°C, 2 min), final elongation at 72°C for 10 min.

PCR name	Primer name	Primer sequence (5`-3`)	Primer type	Target AdV	Target gene	Position in recognized AdV isolate (Genbank accesion N°-AdVtype:genome position)	Annealing temperature (C°)	Product length (bp)	Designed by
DPOL PCR	4431s	GTNTWYGAYATHTGYGGHATGTAYGC	genus-specific degenerate consensus	MastAdV, mainly human and simian Adv	Polymerase gene	AB448769-HAdVD: 6528-6553	45	955	(Wellehan *et al.* 2004)
	4428as	GAGGCTGTCCGTRTC(N/I)CCGTA				AB448769-HAdVD: 5552-5572			(Wevers *et al.* 2011)
	4428s	CGGACGCCTCTGYTGGAC(N/I)AA				AB448769-HAdVD: 6294-6314	45	613	
	4429as	GGCCAGCACrAA(N/i)GArGC				AB448769-HAdVD: 5663-5680			
HEX-HAdVD PCR	6075s	AAGGCCGTCACCCTGCCCTT	species-specific non-degenerate	HAdV C	Hexon gene	AB448769-HAdVD: 20188-20207	63	314	(Hoppe *et al.* 2015)
	6075as	GTGCGGCTGGTGCACTCTGA				AB448769-HAdVD: 20522-20541			
	6076s	ACAACTCGGGCTTCACCGGC				AB448769-HAdVD: 20216-20235	63	281	
	6076as	GGCTGGTGCACTCTGACCACG				AB448769-HAdVD: 20517-20537			
HEX-HAdVE PCR	6070s	CTTCCAGCCCATGAGCCGCC	species-specific non-degenerate	HAdV E	Hexon gene	NC_003266-HAdVE: 20590-20609	64	362	(Hoppe *et al.* 2015)
	6070as	GGCTGGTGCACTCGGACGAC				NC_003266-HAdVE: 20972-20991			
	6071s	GGACTACCAGGCCGTCACCCT				NC_003266-HAdVE: 20635-20655	64	266	
	6071as	AGGGTGGACTCATCCATGGGGT				NC_003266-HAdVE: 20922-20943			
LD-HEX-HAdVD PCR	6146s	CGCCCAGCAATAACACCGGC	species-specific non-degenerate	HAdV D	pV-Hexon gene	AB448769-HAdVD: 15106-15125	60	5396	
	6146as	GTGCGGCTGGTGCACTCTGA				AB448769-HAdVD: 20522-20541			
	6147s	GGCAGGACTCGCAGACGAGC				AB448769-HAdVD: 15537-15556	60	4911	
	6147as	GGTGGGCTCATCCATGGGGT				AB448769-HAdVD: 20468-20487			
	6148s	GCGCGGAAACGTGTACTGGGT				AB448769-HAdVD: 15602-15622	60	4724	
	6148as	GGGATGCGCCACATGACCCT				AB448769-HAdVD: 20347-20366			

Table 8. Description of the applied PCR systems

PCR name	Primer name	Primer sequence (5`-3`)	Primer type	Target AdV	Target gene	Position in recognized AdV isolate (Genbank accesion N°-AdVtype:genome position)	Annealing temperature (C°)	Product length (bp)	Designed by
Animal-HEX PCR	6500s	CGCAGTGGKCNTWCATGCACAT	genus-specific degenerate consensus	MastAdV	Hexon gene	AC_000001-ovAdVA: 16650-16671	56	292	
	6500as	GTGCCGGTGTANGGYTTRAA				AC_000001-ovAdVA: 16964-16983			
	6501s	ACCCACGAYGTSACNACNGA				AC_000001-ovAdVA: 16784-16803	56	160	
Blocking-HAdVD PCR	6542s	CGCAGTGGGCGTACATGCACATCGCCGGGCAGGACGCC TCG--C3spacer	Blocking primer	Blocking primer for HAdV D	Hexon gene	AB448769-HAdVD: 17798-17838	56		
	6543s	ACCCACGATGTGACCACGGACCGGTCCCAGCGT--C3spacer				AB448769-HAdVD: 17932-17964			
LD-HEX-MastAdV PCR	6676s	ATGGCKACSCCKTCGATG	genus-specific degenerate consensus	MastAdV	Hexon gene	AC_000001-ovAdVA: 16628-16645	45	2690	(Lehmkuhl & Hobbs, 2008)
	6676as	GGTRGCGTTSCCGGCBGA				AC_000001-ovAdVA: 19336-19353			
	6677s	GATGMTGCCGCARTGGTC				AC_000001-ovAdVA: 16642-16659	45	2673	
	6677as	CGTTSCCGGCBGAGAA				AC_000001-ovAdVA: 19333-19348			
short HEX-FAdV PCR	6569s	CCTTCTTTTAAACCnTACkGnGGmAC	species-specific degenerate	FAdV	Hexon gene	NC_000899-FAdVD: 20309-20334	45	390	
	6569as	CGAGGCGCTAWAkTCYTCNAC				NC_000899-FAdVD: 20726-20746			
	6570s	GGAGGAACSGCNTAYAAYCC				NC_000899-FAdVD: 20327-20346	45	306	
	6570as	AAGGAGTGGGGTTAADNGAYTGNGA				NC_000899-FAdVD: 20654-20678			
long HEX-FAdV PCR	6708s	CAARTTCAGRCAGACGGT	species-specific degenerate	FAdV	Hexon gene	NC_000899-FAdVD: 20107-20124	54	862	(Meulemans et al., 2001)
	6708as	TAGTGATGMCGSGACATCAT				NC_000899-FAdVD: 20987-21006			
	6709s	SKCSACYTAYTTCGACAT				NC_000899-FAdVD: 20269-20286	54	547	
	6709as	TTRTCWCKRAADCCGATGTA				NC_000899-FAdVD: 20834-20853			
DPOL-PanAdV PCR	6812s	TNMGNGGNGGNMGNTGYTAYCC	family-specific degenerate consensus	MastAdV, SiAdV, AtAdV,AviAdV	Polymerase gene	AC_000001-ovAdVA: 5982-6003	46	508	(Wellehan et al., 2004)
	6812as	GTDGCRAANSHNCCRTABARNGMRTT				AC_000001-ovAdVA: 5448-5473			
	6813s	GTNTWYGAYATHTGYGGHATGTAYGC				AC_000001-ovAdVA:5922-5947	46	275	
	6813as	CCANCCBCDRTTRTGNARNGTRA				AC_000001-ovAdVA:5624-5646			

Table 8 continued.

PCR name	Primer name	Primer sequence (5`-3`)	Primer type	Target AdV	Target gene	Position in recognized AdV isolate (Genbank accesion N°-AdVtype:genome position)	Annealing temperature (C°)	Product length (bp)	Designed by
ruminant-HEX PCR	6856s	TGATCCTTACTTTACTTATTCDGGCACT	ruminant-specific degenerate	ovine and caprine AdV	Hexon gene	AC_000001-ovAdVA: 18583-18610	61	394	
	6856as	GTAAGGGTGGCCTTCYCTDGGYA				AC_000001-ovAdVA: 19005-19027			
	6857s	AGTGTCTATTCAGTTTGAYTCTTCDGTWCA				AC_000001-ovAdVA: 18658-18687	61	286	
	6857as	GTTACCAAAAGCDGTAAAKCCDGA				AC_000001-ovAdVA: 18974-18997			
SiAdV-HEX-PCR	6874s	GTGGAATCAAGCTGTAGATGATTAYGA	species-specific degenerate	TAdV	Hexon gene	AC-000016-TAdV3: 14628-14654	54	538	
	6874as	AACCACCATACATAACTATARGTNCC				AC-000016-TAdV3: 15193-15218			

Table 8 continued.

Electrophoresis and sequencing

PCR products were visualized with gel electrophoresis on 2 % agarose gels, whereas for PCR products obtained with Long-Distance PCR 0.7 % agarose gels were used. DNA fragments were stained with GelRed Nucleic Acid Stain. AdV PCR products of correct size were purified using the purification kit MSB® Spin PCRapace (Stratec Molecular) and sequenced using the Big Dye Terminator v3.1 system (Life Technologies, Grand Island, NY) on an ABI PRISM 3730xl capillary sequencer (Life Technologies, Grand Island, NY). If the gel revealed the presence of several fragments, only those of expected size were excised from the gel, purified using the gel extraction kit, Invisorb® Spin DNA Extraction Kit (Stratec Molecular) according to the manufacturer`s instructions, and used as templates for sequencing.

3.7 Phylogenetic analysis

The phylogenetic analyses did not only elucidate phylogenetic relationships and taxonomic classification of the study sequences, but were also used to unravel potential disease transmission routes and to detect hints for recombination events.
Prior to phylogenetic analysis, sequence reads were pre-processed by trimming primer sequences, as well as regions of the chromotagrams that were considered of low quality. They were then assembled in Geneious 7.1.4. (Kearse *et al.* 2012), and consensus sequences were derived.For every consensus sequence, a BLAST analysis was performed to confirm its origin. These validated sequences were added to a data set comprising homologous and related AdV sequences available in Genbank and aligned with the ClustalW multiple alignment method (Thompson *et al.* 1994). To each alignment an assumed outgroup sequence was added to permit correct tree rooting. In the process of aligning sequences, gaps are included in locations, where base deletion or insertion occurred. After aligning, the nucleotides or amino acids in one column of the matrix should ideally all be derived from the same ancestral position in the according genome ("positional homology"). As it has been shown that the removal of poorly aligned regions from an alignment increases the quality of subsequent analyses, conserved blocks from the alignment were selected, using Gblocks online (Castresana 2000, Talavera *et al.* 2007) or as implemented in SeaView v4 (Gouy *et al.* 2010).

For every alignment the best-fit model of nucleotide substitution given the data was selected in a maximum likelihood framework using jModelTest v2.1.4 (Darriba *et al.* 2012). It is indeed well-known that observed genetic differences between sequences is not necessarily a reliable indication of the amount of substitutions that they accumulated since their divergence. By selecting the best-fit model of nucleotide substitution the evolutionary process can be estimated more accurately. Every model of evolution includes a set of assumptions about the process of nucleotide substitution in order to correct for hidden modifications along the phylogeny. In the most complex model, GTR (Generalised time-reversible) (Lanave *et al.* 1984, Rodriguez *et al.* 1990), different instantaneous mutation rates are assumed for all 6 possible bidirectional changes (A<>T, A<>C, A<>G, T<>C, T<>G and C<>G; multiple-parameter model), whereas in the simplest one, JC (Jukes and Cantor)(Jukes and Cantor 1969) the same probability is assigned to each kind of event (single-parameter model). It is important to incorporate only as much complexity as needed, since, although complex models improve the fit to the data, they come with the difficulty that accurate parameter estimates are harder to generate (e.g. require more information) and are computationally intensive. Model selection strategies attempt to find the appropriate level of complexity on the basis of the given data. In the present study the Akaike and Bayesian information criteria (AIC and BIC) were considered

together as model selection strategies (Akaike 1974, Schwartz 1978): the model with the smallest AIC and the best BIC support was selected for further phylogenetic analyses. Finally, Bayesian, as well as maximum-likelihood phylogenetic approaches were used as statistical inference methods. Their results were systematically compared and consistency across methods was considered necessary to validate an evolutionary hypothesis. The evolutionary relationships were represented on rooted phylogenetic trees. In the maximum-likelihood inference, the tree with the highest likelihood is identified and considered as the best estimate: meaning the tree with the highest probability of producing the observed data, considering the model (i.e. substitution model+tree). This best tree is referred to as the maximum likelihood (ML) tree. The underlying logic of Bayesian analysis is very different. In the first place, Bayesian analyses aim at assessing the probability of the model given the data (i.e., it is the reciprocal of likelihood). In addition, as there is no way to calculate posterior probabilities analytically, even for a single tree, specific algorithms called Markov chain Monte Carlo (MCMC) samplers are used to approximate them. The process implies that the end result of the analysis is a large sample of values for all parameters of the model (including trees) rather than a point estimate. It should be noted that both inference methods are closely related as the posterior probability of a hypothesis is proportional to the likelihood multiplied by the prior probability of that hypothesis.

In order to estimate the branch support, and to assess the accuracy of the best tree obtained with the two phylogenetic approaches, bootstrapping can be applied for the maximum-likelihood inference. For the Bayesian approach, the Markov-chain Monte Carlo results in a pool of plausible trees. With the bootstrapping method, the pool of trees is obtained by reconstructing trees based on pseudo-replicate data sets generated by randomly re-sampling sites. Estimation of the branch support is then based on the proportion of bootstrap trees showing a same clade. From the resampled trees, a consensus tree can be obtained viewing this proportion or bootstrapping value for every clade. With the Markov chain Monte Carlo (MCMC) method, the pool (sample) of trees is obtained by generating new trees from a random initial tree by changing model parameters or tree topology. Branch support is expressed as posterior probability. From the resampled trees, a consensus tree can be obtained and used to plot branch posterior probabilities (alternatively a tree thought to be a good representative of the sample can also be chosen for the same purpose).

Maximimum Likelihood analysis

Maximum likelihood phylogenies from the study alignments of nucleotide or amino acid sequences were estimated using the PhyML online web interface. The PhyML (PHYlogenetic inferences using Maximum Likelihood) software implements the heuristics described by Guindon and Gascuel (Guindon *et al.* 2003, Guindon *et al.* 2005). For each phylogenetic analysis, the input data consisted in the respective nucleotide or protein alignment alignments in PHYLIP format. In the first step, the model previously selected with jModelTest v2.1.4 or Prottest (Abascal *et al.* 2005, Darriba *et al.* 2012) would be chosen. The type of tree improvement was always set to SPR&NNI (BEST). In this case 2 distinct hill-climbing algorithms are used to generate new tree proposals (Nearest Neighbor Interchange, NNI, and Subtree Pruning and Regrafting, SPR), ensuring the tree space will be explored thoroughly. Each analysis was started with 5 random trees. Both topology and branch length were optimized in order to maximize the likelihood. The reliability of internal branches was assessed using nonparametric bootstrap with at least 500 boostrap pseudo-replicates. Results, including among

others the maximum likelihood phylogeny in NEWICK format, were obtained by electronic mail. The phylogenetic tree was then viewed and processed in Figtree (Rambaut *et al.* 2014).

Bayesian Analysis

Bayesian phylogenies from the study alignments of nucleotide or amino acid sequences were estimated using the Bayesian Evolutionary Analysis by Sampling Trees (BEAST) software package version 1.8 (Drummond *et al.* 2012, Bouckaert *et al.* 2014), which implements a family of Markov chain Monte Carlo (MCMC) algorithms (Metropolis-Hastings MCMC) for Bayesian phylogenetic inference, divergence time dating, coalescent analysis, phylogeography, and related molecular evolutionary analyses.

For each phylogenetic analysis, the input data consisted in the respective nucleotide or protein alignment in FASTA format. These data were imported into the graphical user interface (GUI) for BEAST, Bayesian Evolutionary Analysis Utility (BEAUti). After selecting the model, choosing the prior distributions on individual parameters, and specifying the settings for the MCMC sample, BEAUTi generates the XML input format for BEAST. In the model previously selected with jModelTest v2.1.4 was chosen, and the Markov Chain Monte Carlo algorithm was run in BEAST (Drummond *et al.* 2012, Bouckaert *et al.* 2014). A relaxed log-normal molecular clock was chosen as model of rate variation among branches. This model does not assume a constant evolution rate across lineages and estimates the rate at each branch from a log-normal distribution. The prior assumption of a constant population size throughout the time spanned by the genealogy (coalescent likelihood) was specified. 2-3 independent and parallel Markov Chain Monte Carlo were run simultaneously with a chain length of 20, 000, 000 steps and a sampling every 1000 steps. The convergence of Bayesian analyses was assessed using Tracer v1.5 (Rambaut A *et al.* 2009), a graphical tool for MCMC output analysis. In Tracer, the posterior distributions of the model parameters estimated during the MCMC runs were visualized. Moreover, one could control if these values reached stationary distribution (trace history forming a'fuzzy caterpillar') and had sufficiently large effective sample sizes (ESS) (combined ESS of >200). In fact, in a first phase the likelihoods of the model increases rapidly and reaches the plateau (stationary phase) as values of all model parameters converge to their range of plausibility. This first phase is called burn-in and is discarded in the subsequent analyses. The convergence diagnostics suggested that the MCMC runs converged by approx. 2000,000 steps. These first 2000,000 steps were discarded as burn-in and the tree and log files obtained from the 3 MCMC runs were combined using logcombiner resampling every 10, 000g. A consensus ultrametric tree (or chronogram) was generated using Treeannotator and was written in either NEWICK or NEXUS format. Note that in a chronogram all current taxa are equidistant from the root (i.e. branch lengths are directly proportional to time). The phylogenetic tree was then viewed and processed in Figtree (Rambaut *et al.* 2014).

Phylogenetic analysis performed

As sequences from different AdV genes and from AdV of different genera and animals were obtained, diverse phylogenetic analyses had to be performed by applying the approach described above *(Table 9)*. For every analysis, a Bayesian and a maximum-likelihood tree were constructed and compared. As both inference methods resulted in similar trees, only the Bayesian trees are depicted in the thesis.

Name of tree	PCR applied	Number of taxa	Lenght of alignment	Evolution model selected	Amount of Boostrap replicates	Figure N°; page
Phylogenetic tree of Mastadenovirus	LD-HEX-MastAdV PCR	39	1856	GTR+I+G	1000	10; 85
Phylogenetic tree of Simian AdV	LD-HEX-MastAdV PCR	29	2043	GTR+I+G	1000	11; 87
Phylogenetic tree of Adenoviridae	DPOL-PanAdV PCR	52	191	HKY85+I+G	1000	12; 91
Phylogenetic tree of the FAdV	long HEX-FAdV PCR	57	469	HKY85+G	1000	14; 96

Table 9. Description of the phylogenetic analyses

Molecular identity

There are two approaches for the comparison of molecular identity of sequences: comparison of observed genetic distance and comparison of estimated genetic distance, i.e. evolutionary distance. The genetic distance is the quantitative measure of genetic divergence between two sequences. With Geneious 7.1.4., observed genetic distances can be retrieved directly from any alignment of homologous sequences. In the obtained observed genetic distance matrix, the genetic distance is the proportion of sites that differ between two sequences, or in other words the observed number of nucleotide substitutions per site.

A serious drawback of observed genetic distances is that they are not an accurate reflection of the pairwise evolutionary distance as they do not take into account that, over time, multiple substitutions may affect a same site. If only the observed distances are considered, back mutations and serial substitutions, but also varying rate variations among sites are disregarded, leading to potential false interpretation of the genetic distances (which are often discussed in an evolutionary framework). Estimated genetic distances, or evolutionary distance, take such unobserved changes into consideration and are thus a more accurate way to describe the evolutionary process as they model substitutions. Moreover, they can also take into account rate variation across sites and nucleotides, etc. With this approach, the number of nucleotide substitutions per site is estimated from the alignment based on the assumptions about substitution rates of a specific evolutionary model. They can be estimated in PAUP* v4.0 (Swofford 2003)and are based on the evolutionary model determined for the respective alignment by model selection programs, such as jModelTest v2.1.4 (Darriba *et al.* 2012).

➔ **Analysis of genetic distances of HAdV D detected in humans**

The HAdV D sequences detected in human and animal study participants (n = 13) were added to a data set consisting of sequences of the hexon gene sequences available in Genbank from completely sequenced HAdV D genomes (n = 43) (listed below). They were aligned with the ClustalW multiple alignment method (Alignment 1) (Thompson *et al.* 1994). Another alignment was performed comprising only the 43 Genbank sequences (Alignment 2). In addition, the hyper-variable loop regions of the hexon gene were extracted from alignments, resulting in the alignments 1a and 2a. Conserved blocks were selected from these alignments, using Gblocks (Talavera *et al.* 2007) as implemented in SeaView v4(Gouy *et al.* 2010). This resulted in alignments of 4.556 (Alignment 1 and 2) and 1.065 nucleotides (Alignment 1a and 2a). Observed genetic distance matrices were obtained for every alignment with the program Geneious v6.1.6 (Kearse *et al.* 2012). Evolutionary distances were estimated using PAUP* v4.0 (Swofford 2003), based on the best fitting nucleotide substitution model (GTR + I + G), as determined using jModelTest v2.1.4 (Darriba *et al.* 2012).

In the observed and estimated genetic distance matrices of the alignments 1 and 1a, the minimum genetic distance (minGD) was determined for every sequence of this study (minGD study 1-13) in relation to the 43 HAdV D types from Genbank. Correspondingly, in the observed and estimated genetic distance matrix of alignment 2 and 2a the minGD was defined for every HAdV D type from Genbank (minGD Genbank 1-43) in relation to the other 42 types. Subsequently, the 13 observed minGD study values and the 43 observed minGD Genbank values were visualized in a strip chart {carried out with R software (R-Core-Team 2014)} (*Figure 7)* and assessed for every minGD study value the number and percentage of inferior minGD Genbank values (*Table 13*).

Genbank accession numbers of published adenovirus sequences used for comparison of genetic distances. Genbank accession numbers AB448769; AJ854486; JN226746; JN226747; AB562586; AF108105; AB448774; JN226749; FJ404771; JN226750; JN226751; JN226752;EF153474; JN226753; FJ824826; AB562587; JN226755; JN226756; JN226758; GQ384080; DQ393829; AB448778; JN226759;JN226760; JN226761; JN226762; JN226763; JN226764; AY875648; JN226757; EF153473; JN226765; AB605240; AB333801; HM770721; HQ883276; JF799911; JN162672; JN162671; JN935766; JQ326208; AP012285; AP012302.

Genbank accession numbers of adenovirus sequences detected in humans in this study.

hu4108_DRC : KF976521; hu4109_DRC: KF976522; hu4214_DRC: KF976523; hu4152_DRC: KF976524; hu4719_IC: KF976525; hu4746_IC: KF976526; hu4751_IC: KF976527; hu4787_IC: KF976528; hu4806_IC: KF976529; hu4813_IC: KF976530; hu4882_IC: KF976531; hu4555_UG: KF976533; hu4557_UG: KF976532; hu4564_UG: KF976534

➔ **Analysis of genetic distances of HAdV D detected in animals**

The HAdV D detected in animals were analyzed with an approach comparable to that applied on the HAdV D detected from the humans. The HAdV D detected in animals (n=6) were added to the dataset comprising the HAdV D from humans previously analyzed and from Genbank (*3.7 Phylogenetic analysis "Analysis of genetic distances of HAdV D detected in humans").* Again, observed and estimated genetic distance matrixes were obtained from a gblocked alignment of 1779 bp and the minGD was determined for every sequence in relation to the 43 HAdV D types from Genbank. However, this time the hypervariable Loop region was not analyzed separately. Subsequently, the 6 estimated minGD study values obtained for the animals, the 13 estimated minGD study values obtained for the humans and the 43 estimated minGD Genbank values were visualized in a strip chart {carried out with R software(R-Core-Team 2014)} (*Figure 9*).

Genbank Accession Numbers of study sequences: DGAH350_HAdVD: KP274040; DGOU241_HAdVF: KP274041; DGOU263_HAdVD: KP274042; DKEI526_HAdVD : KP274043; DPON033_HAdVC: KP274044; GGAH386_caAdV2: KP274045; GTAI086_HAdVD: KP274046; GZAI440_caAdV2: KP274047; MTAI277_SAdV: KP274048; PGAH389_HAdVD: KP274049; PGAH389_PAdV: KP274050; PGOU244_PAdV3: KP274051; PKEI502_PAdV3: KP274052; SPAU316_ovAdV4: KP274053

➔ **Analysis of genetic distances of FAdV**

The approx. 500 bp long sequences determined in this study (n=22) were added to a data set that comprised sequences of at least one FAdV isolate of every recognized FAdV serotype (n = 41) (listed below). They were aligned with the ClustalW multiple alignment method (EMBL, Heidelberg, Germany). Conserved blocks were selected from these alignments, using Gblocks (Talavera *et al.* 2007) as implemented in SeaView v4 (Gouy *et al.* 2010). This resulted in alignments of 459 nucleotides. With the program Geneious v6.1.6 (Kearse *et al.* 2012), observed genetic distance matrices were obtained. For every study sequence the maximum observed genetic distance compared to the recognized FAdV serotypes was retrieved from the matrix. FAdV type and species of the study sequences were identified based on the threshold values for pairwise sequence identity proposed by Marek et al, 2010 (Marek *et al.* 2010) (nucleotide sequence identity of approx. 72 % for species and of 95 % for type identification) (*Table 16*).

Genbank Accession Numbers of study sequences: FAdVD_CDAO107: KP274018; FAdVD_CKEI522: KP274019; FAdVD_CKEI519: KP274020; FAdVD_CZAI444: KP274021; FAdVD_CDAO120: KP274022; FAdVD_CDAO164: KP274023; FAdVE_CGOU266: KP274024; FAdVE_CGOU267: KP274025; FAdVE_CGOU234: KP274026; FAdVE_CPON002: KP274027; FAdVE_CDAO197: KP274028; FAdVB_CGOU223: KP274029; FAdVE_CPAU281: KP274030; FAdVE_CZAI462: KP274031; FAdVE_CPON012: KP274032; FAdVE_CZAI445: KP274033; FAdVB_CGOU224: KP274034; FAdVB_CPON047: KP274035; FAdVB_CPAU286: KP274036; FAdVB_CPON040: KP274037; FAdVE_CDAO182: KP274038; FAdVC_CDAO110: KP274039;

Genbank accession numbers of published adenovirus sequences used for comparison of genetic distances. FN869991; FN869990; AF339916; AF339922; AF508955; AF339920; AF508954; FN869966; FN869968; EF685482; AF508957; FN869964; FN869965; AF508958; AF339919; AF339924; FN869959; FN869960; FN869958; EF685611; AF508959; AF508946; FN869963; EF685529; AF339915; AF339921; AF339918; AF508953; FN869971; FN869969; FN869972; AF508950; FN869975; FN869977; FN869976; FN869978; AF154246; AF339923; FN869984; FN869986; AF339914

Species delineation analysis

The Generalized Mixed Yule Coalescent (GMYC) method was applied on the alignment of FAdV sequences used for the analysis of genetic distance (see above), after selecting all unique sequences of the alignment (removing all duplicate sequences) with Fabox (Villesen 2007).
In a first step, a Bayesian MCMC analysis of an alignment of 58 discrete FAdV sequences was performed under a relaxed lognormal molecular clock in Beast v.1.8.0 (Drummond *et al.* 2012, Bouckaert *et al.* 2014) with the evolutionary model HKY+G previously selected with jModelTest v2.1.4 (Darriba *et al.* 2012). The prior assumption of a constant population size throughout the time spanned by the genealogy (a coalescent model) was specified. 2 independent and parallel MCMC were run simultaneously with a chain length of 20 Mio steps and a sampling every 1000 steps.

2 independent and parallel Markov Chain Monte Carlo were run simultaneously with a chain length of 20 Mio steps and a sampling every 1000 steps. The convergence of Bayesian analyses were assessed using Tracer v1.5 (Rambaut A *et al.* 2009). The combined ESS-values were > 200 for every parameter and the first 5 Mio generations were discarded as burn-in. The log- and tree-files generated in BEAST in the two runs were combined with Log Combiner with a resample states frequency of 10 000 and the burn-in of 5 Mio. A consensus ultrametric tree was selected with Treeannotator using the combined Trees.

Finally, species delineation analysis was conducted in R (R-Core-Team 2014) with the package "splits" (Ezard and Fujisawa and Barraclough 2013). The statistical method applied, the Generalized Mixed Yule Coalescent (GMYC) was devised to determine the most likely species delimitation using single locus data. The null model to be rejected by the method is that all sequences belong to a single species; the alternate model is that the sequences belong to different species. The ML-based determination of a "threshold time", representing the precise location of inter-species nodes, permits to distinct between within- and between-species branching patterns and thus also to assign lineages to putative species. Nodes before the threshold time are considered to stand for speciation events and nodes after the threshold time coalescent events (Fujisawa and Barraclough 2013).

In the present study, the number of delimited species was identified with the single- and the multiple-threshold approach. These two methodological variants either use a single threshold applied to the entire tree or allow for multiple thresholds in different locations of the tree. Contrary to the multiple-threshold approach, the single-threshold approach therefore assumes that inter-species nodes are always older than within-species nodes. The multiple-threshold method is less conservative than the single-threshold method. Although simulations revealed that this method tends to erroneously identify more species than the single-threshold method, it seems to still be sufficiently conservative. For each approach, a p-value is given, which estimates the probability of wrongly rejecting the null hypothesis. A significant p-value ($p<0.05$) means that the sequences belong to different species (according to the analysis) (Fujisawa *et al.* 2013).

By comparing the maximum likelihoods of the GMYC models and by interpretating the p-values of the single- and multiple-threshold approach, the most likely species delineation can be statistically identified. Moreover, support values (Akaike information criterion weights) can be obtained for each genetic cluster identified as distinct species.

3.8 Recombination analysis

In the phylogenetic analyses it was assumed that recombination did not affect the data set under consideration (Posada *et al.* 2002). One way to evidence recombination is to show multiple trees, based on various genome regions. On such trees, conflicting phylogenetic relationship of viruses in different genome regions suggests recombination events. Alternatively, other methods can be employed that will allow for the detection of recombination breakpoints (without having to define putative recombination breakpoints *a priori*).

The Recombination Detection Program v.4.16 (RDP4) (Martin *et al.* 2005, Martin *et al.* 2010) includes a number of statistical, non-parametric recombination detection and analysis methods. Contrary to most other recombination detection programs, the input file of RDP4 is a nucleotide alignment. Every sequence of an alignment is considered as a potential recombinant and by pairwise scanning

approach resembling parent sequences are identified. The proposed, statistically supported, recombination events are visualized graphically and by means of phylogenetic trees constructed from proposed recombinant regions. Moreover the program notifies if the proposed recombinant is only badly supported. Potential recombination events, likely parental strains of recombinants and recombination hotspots were analyzed using the RDP, Geneconv, Bootscan, MaxChi, Chimaera, SiScan, 3Seq, LARD and TOPAL methods implemented in RDP4.

3.9 Statistical analysis

Statistical analysis mainly aimed at describing the factors influencing AdV shedding in human and animal hosts. Moreover, the occurrence of behaviours that pose a potential risk for zoonotic transmission of pathogens was determined. First, basic descriptive statistics of the datasets were performed (calculation of the mean, confidence intervals, and percentage/prevalence) in R (R-Core-Team 2014).

➔ Statistical analysis of the epidemiology of HAdV D in 4 sub-saharan countries (Pauly *et al.* 2014)

Descriptive statistics, prevalence estimation and the effects of demographic data were generated and analyzed in Stata v12.0, using cross tables with Fischer exact tests and logistic regression models in Stata v12.0 with HAdV D status as dependent binomial factor and country, gender, age group and village as independent factors. Results from CAR and UG were used for prevalence estimation only *(Table 12)*.

➔ AdV shedding in mammals of Côte d`Ivoire

In order to analyse which factors affect AdV shedding, we analyzed the data using a Generalized Linear Mixed Model (GLMM) with binomial error structure and logit link function (Baayen 2008, Bolker *et al.* 2009). The categorical predictors included as fixed effects were animal species (dog, sheep and goat), sex (F/M), and age (adult/juvenile). Sampling village (Taï, Ponan, Daobly, Gahably, Zaipobly, Keibly, Pauleoula and Gouleako) was included as random effect (Barr *et al.* 2013). Only the data collected and obtained for dogs, sheep and goat were included in the dataset for the statistical analysis as too few cows (only from few villages), pigs (only samples from juveniles and from few villages) and monkeys (only from few villages) were tested. Including them into the model would have probably led to the occurrence of influential cases and leverage issues, due to an imbalanced data set.

It was hypothesized that AdV shedding might be diet-related and it was attempted to elucidate if there is a significant difference between herbivore (goat and sheep) and omnivore (dog) species; in particular, as there are considerable disparities in digestion duration between the two diets. Many dogs are kept as hunting dog and are frequently in close contact with body fluids and tissues of wildlife. Thus dogs are certainly the animal species with close contact to most different potential AdV hosts. Age was included as predictor as the aim was to illuminate whether, similar to humans (Pauly *et al.* 2014), juvenile animals shed AdV more often and in higher quantity than adult animals. For humans, there was no significant difference in AdV shedding between sexes. Nevertheless, we included sex as predictor firstly to compare the animals to the humans; secondly as it is probable that recurring gestation and lactation periods might affect AdV shedding.

A random effect is “*an error term that captures the variability of the effect across individuals*” (Barr *et al.* 2013). Hence by controlling for the random effect sampling village, one accounts for the varying effect *Sampling village* might have on AdV shedding. As matter of fact, depending on the available water sources, the proximity to the National Park, the size of the village, etc. the effect of *Sampling village* on AdV shedding might change. In contrast fixed effects (age, sex and animal species) are “*effects that are assumed to be constant from one experiment to another*” (Barr *et al.* 2013). The results of the Animal-HEX PCR were taken as response and thus the response was binomial with two possible levels: presence or absence of AdV in the animal rectum swabs. This was the reason, why we had to apply a GLMM, which allows modelling with error distributions other than normal distribution and includes fixed and random effects. In a binomial error distribution the model predicts the probability of “success” (here: presence of AdV). In a GLMM, the linear predictor (LP) does not always equal to the fitted value (FV).

The assumptions of a GLMM are largely the same as those of multiple regression and identical with regard to the predictors (absence of influential cases, absence of collinearity, etc.). An additional assumption of logistic models is the absence of overdispersion. Before calculating a GLMM, all assumptions were tested in order to obtain a reliable and stable model. To establish the significance of the full model the likelihood ratio test was used. It compars the deviance of the full model with that of the null model comprising only the random effect. If deviance is the measure of how well a model fits, the 'likelihood ratio' test statistic is defined as the difference between the deviances of two models.

LR=deviance_reduced - deviance_full

By this calculation, one can assess whether the predictor(s) have explanatory value and at the same time, one can establish the significance of the full model compared to the null model. No R^2-like effect size can be calculated for the full GLMM, but one can get a marginal R^2, which is the variance explained by the fixed effects and a conditional R^2, which is the variance explained by the model (Nakagawa and Schielzeth 2013).

In a second approach, the potential association between AdV shedding and health status was elucidated. We assumed that nutritional condition and body temperature were well adapted to describe the current health status (e.g.high body temperature or moderate nutritional condition associated with bad health status). The results of the Animal-HEX PCR were again taken as response and thus the response was binomial with two possible levels: presence or absence of AdV in the animal rectum swabs. Temperature was included as covariate and nutritional condition as categorical predictors. The data were analyzed as described above using a GLMM with binomial error structure and logit link function.

The models were fitted in R (R-Core-Team 2014) using the function glmer of the R-package lme4 (Bates *et al.* 2014). We tested for model stability by deriving the Variance Inflation factor using the function vif of the R-package car (Fox and Weisberg 2011) applied to a standard linear model excluding the random effects. The likelihood ratio test was performed by application of the function anova with argument test set to “Chisq”. The individual effects were based on likelihood ratio tests comparing the full with respective reduced models (R function drop 1) (Barr *et al.* 2013).

➔ Descriptive statistical analysis of the questionnaires: activities implicating contact to animals

We considered that every contact to tissues or body fluid constitutes a risk for pathogen transmission and that this risk is even increased if the animal is still alive (risk to get bitten, scratched) and if the meat and tissues are raw. For the data on contact to domestic animals, we limited the analysis to the descriptive statistical analyses of the data obtained from the 160 adults and adolescents, whose fecal samples were also tested for AdV. However, all available data on contact with live and dead NHP were analyzed, as activities implicating contact to NHP were considered to be the major risks for zoonotic transmission of pathogens. The dataset on contact with NHP was analyzed with descriptive statistics and with a GLMM. Some study participants were reluctant to answer some of the questions, thus the questionnaires of some were incomplete.

It was hypothesized that contact to NHP bushmeat might depend on ethnicity, birthplace, sex and place of residence (equaling the sampling village). The complexity and the numerous combinations of origin of migration, ethnicities and residence complicated accurate statistical analyses. Therefore we simplified the dataset by replacing birth-villages by birth-country (*Birthplace: Country*) and the ethnicity by the country comprising the territory of the specific ethnic group (*Ethnicity: origin*). As the territory of the ethnic group "Senoufo" is divided between CI and BF, this ethnic group was referred to as "CI/BF". For most ethnic groups the country assignment was less amibiguous (e.g. Mossi to BF and Guéré to CI). If the dataset comprehended only few cases (e.g. people with *Birthplace: Country* Mali *or Ethnicity: origin* Ghana) such cases were referred to as "others".

Birthplace might play a role for bushmeat consumption, as it is probable that people, born in the Sahel or dry savanna zones (e.g many regions from BF) and without easy access to bushmeat, have other food habits than people born in close neighborhood to the tropical forest (e.g. many regions in CI). Moreover, it might be interesting to analyse whether these nutritional habits change depending on the living place (e.g. after migration). Ethnicity was also considered in the descriptive statistics about contact to NHP bushmeat, as *Birthplace: Country* did not always equal *Ethnicity: origin.* Hence, many people, which were born in CI and with Burkinabé origins (second-generation immigrants), claimed to belong to an ethnical group from BF. Religious faith is closely linked to ethnic group (e.g. majority of Muslims in ethnic groups from Burkina Faso and of Christians in ethnic groups from Western CI) and the religion might further influence the nutritional habits (e.g. Muslims do not eat pigs). Unfortunately, no data were available on religion and hence no definite relation could be made between religion and ethnicity. We assumed that the religious faith and the ethnic group might also influence the differing nutritional habits between men and women, particularly concerning bushmeat consumption. It is conceivable that in male-dominated societies, luxury food, as for example bushmeat, might be reserved to men.

We also considered sample location or place of residence (*Village*), as although all study villages are situated in the same region, there might be differences in the bushmeat consumption. In fact, bushmeat consumption might depend on the ethnic group composition of the village, or on the distance to the Taï National Park or to Guiglo (next bigger city). However the effect was considered to be minimal, as all the villages are situated along the Cavally river (border to Liberia) and on the same logging road, so transportation and trade of bushmeat even from more distant regions should not be an issue. A factor potentially leading to differences between the villages could also be the activities of the locally operating NGOs. For instance, the NGO *Wild Chimpanzee Foundation,* WCF, employs many locals from the villages around Taï village and has organized several awareness campaigns, which may have had an impact on the bushmeat consumption in some villages. However, this might also have affected the validity of the questionnaires, especially since bushmeat consumption is

officially illegal. To reduce the chance of such a bias, the field missions were performed independently from the WCF and from the local authorities (e.g. OIPR).

Other predictors potentially influencing nutritional habits were not available or not considered: e.g "totems" (an animal species, which serves as emblem for a particular ethnical group and which cannot be harmed, as it is considered to be connected to the ancestors of the group) or wild pets (are they rater a status symbol or a future meal?).

Different statistical models were calculated in order to analyze which factors influenced participants "*to eat/dismember/cook/hunt or not to eat/dismember/cook/hunt monkey/chimpanzee*". The data were analyzed using a Generalized Linear Mixed Model (GLMM) with binomial error structure and logit link function (Baayen 2008, Bolker *et al.* 2009). The response was binomial with two possible levels: "*to eat/dismember/cook/hunt or not to eat/dismember/cook/hunt monkey/chimpanzee*".

Before calculating the GLMMs, all assumptions (e.g. absence of influential cases, of multicollineearity and of overdispersion) were tested as this is of paramount importance to obtain a reliable and stable model. The data collected in Taï were not included into the model, as in Taï only women working in the local restaurants were questioned. Moreover, other inclusion criteria were "born in Côte d`Ivoire or Burkina Faso" and "ethnic group of one of these two countries". The final complete dataset comprised the data of 378 participants. The categorical predictors included as fixed effects were *Birthplace: Country or Ethnicity: Origin* (CI/BF), and sex (F/M), and age group (1:1-20, 2:21-40, 3:41-60, 4:61-80). Sampling village (Ponan, Daobly, Gahably, Zaipobly, Keibly, Pauleoula and Gouleako) was included as random effect (Barr *et al.* 2013). The general idea of the model was that the categorical predictors (fixed effects) and the random effect influence the behavior of participants with regard to NHP bushmeat contact. To establish the significance of the full model we used a likelihood ratio test, comparing its deviance with that of the null model comprising only the random effect (*Sampling village*). Then we calculated the marginal and conditional R^2 (Nakagawa *et al.* 2013).

The models were fitted in R (R-Core-Team 2014) using the function glmer of the R-package lme4 (Bates *et al.* 2014). We tested for model stability by deriving the Variance Inflation factor using the function vif of the R-package car (Fox *et al.* 2011) applied to a standard linear model excluding the random effects. The likelihood ratio test was performed by application of the function anova with argument test set to "Chisq". The individual effects were based on likelihood ratio tests comparing the full with respective reduced models (R function drop 1) (Barr *et al.* 2013).

4 Results

The results section is divided into five parts. In the first part (4.1), the risk factor for zoonotic disease transmission observed in the Taï region and retrieved from the questionnaires on exposure to domestic animals and wildlife are exposed. In the following three parts (4.2-4.4), AdV shedding by humans and animals from the study region is presented and the phylogenetic, statistical and recombination analyses described. The last section (4.5) encompasses the results of the analyses of the zoonotic potential of AdV.

4.1 Risk factors for zoonotic disease transmission in rural region of Côte d`Ivoire (Mossoun *et al.* 2015)

Description of the population in the Taï region. Descriptive statistics revealed a high prevalence of participants with immigration background, mainly from Burkina Faso (n=195). Few participants immigrated from Mali (n=24), Benin (1) and Ghana (n=1). In fact, among the ethnic groups observed (>24 different ethnic groups), about 50 % originate from Côte d`Ivoire (the most prevalent groups being Baoulé, Guéré, Malinké and Ubi) and 48 % originate from Burkina Faso (the most prevalent groups being Mossi and Grussi) *(Table 4)*. In some villages (e.g. Daobly and Ponan), more participants with immigration background, than original Ivorians, participated in the study. If they were just more willing to participate in the study or if this proportion really reflects the real situation in these villages could not be elucidated *(Table 5)*. Only around 30 % of the participants reported that they were born in Burkina Faso (around 60 % in Côte d`Ivoire). The majority of the participants were born in the villages bordering the Taï National Park (n Pauleoula=45; n Taï=43; n Gouleako= 34, etc.) *(Table 4)*.

Contact to domestic animals. 108 of the 160 (67.5 %) questioned adults and adolescents keep domestic animals and chickens are the most common animal species bred. None of these animals has ever been treated, vaccinated or vermifuged by a veterinarian. Carcasses from dead animals are mostly consumed (44.9 %) or dumped (42 %). The animal products, e.g. honey, milk and eggs, are processed in the traditional dishes. More than 95 % of the adults and children appeared to eat chickens and ruminant meat, mainly on special occasions. The lower pig meat consumption (less than 50 % of the locals) was justified by personal reasons or traditional or religious customs (e.g. Muslim religious laws). 54 % of the locals slaughter their chickens and process the meat themselves. Ruminants and pigs are butchered in more than 70 % of the cases by vocational slaughters and the meat is then sold on the local market. Besides ruminant and pig meat, fresh or smoked bushmeat (meat from wildlife) is available on the local market. 98 % of the locals declared that they consume and 61.8 % that they cook bushmeat other than primates (e.g. antilopes). While meat of domestic animals is mainly fresh, bushmeat is often smoked to prolong the storage time *(Table 10)*.

	Chickens	Ruminant	Pigs	Bushmeat other than NHP
Question	**% yes**	**% yes**	**% yes**	**% yes**
Eaten by adult?	98.1	98.7	45.3	98.1
Eaten by girls?	97.5	97.5	46.2	98.7
Eaten by boys?	96.8	96.8	46.8	98.7
Do you kill/hunt?	53.6	25.8	10.3	11.8
Do you dismember?	60.1	32.5	18.4	24.0
Do you cook?	65.8	62.7	52.7	61.8
State of the meat:	mainly fresh	mainly fresh	mainly fresh	mainly fresh or smoked
Origin of the meat:	mainly from market, personal breed	mainly from market	mainly from market	mainly from market
If not eaten, why?	Custom or personal	Personal	Custom or personal	Custom or personal
How often do you eat?	occasionally	occasionally	occasionally	occasionally

Table 10. Frequency of exposure to body fluids of domestic animals

Descriptive statistics and GLMM: contact to non-human primates (NHP). Descriptive statistics showed that there were considerable frequency differences in bushmeat-related activities for the factors *Sex, Age group, Village, Birthplace: country* and *Ethnicity: Origin.* There was a clearly assigned role allocation between women and men: while men are responsible for hunting and dismembering, women cook the bushmeat. Another finding was that the older generations (age groups 41-60 and 61-80 years) reported more often to hunt, dismember, cook and eat NHP. When comparing participants born in BF (or belonging to an ethnic group of BF) to particpants from CI (or belonging to an ethnic group of CI) it became clear that latter are more frequently in contact with NHP *(Table 11)*.

Differences between villages were less obvious and depended on the activity and on the NHP species (chimpanzee or monkey): the largest number of people reporting to hunt lived in Keibly (16 % for monkey and 4 % for chimpanzee hunting). Only few people reported to hunt monkeys in Daobly (2.4 %) and Ponan (3.3 %), to hunt chimpanzees in Taï (0 %) and Pauleoula (0%). For dismembering and cooking NHP, a high variation between the villages was revealed, ranging from 10 % in Ponan to 47.4 % in Taï for dismembering of monkey carcasses, and from 0 % in Taï and Daobly to 4 % in Keibly for dismembering of chimpanzee carcasses. On average, 22.1 % of the villagers admitted to dismember monkeys and 3.2 % to dismember chimpanzees. Considering all the activities together, villagers from Taï and Gahably appeared to be most frequently in contact with monkeys and villagers from Zaipobly and Keibly to be most frequently in contact with chimpanzee, irrespective of the exposural activity. However, direct comparison of the data obtained in Taï with those of the other villages is not accurate, as in Taï only women working in the local restaurants (*"maquis"*) were questioned, whereas in the other villages everyone could volunteer to participate in the study. Consumption of NHP meat is still widespread and on average 62.2 % of the villagers appeared to consume monkeys and 18.4 % chimpanzees *(Table 11)*. Moreover, the frequency of monkey contact was substantially higher than the frequency of chimpanzee contact, irrespective of the particular activity. The frequency of people reporting to do a bushmeat related activity increased from few people reporting to hunt, to numerous reporting to eat NHP, irrespective of the NHP species *(Figure 6 and Table 11)*. Most people reported to be in contact with NHP bushmeat during multiple activities (e.g. while hunting and dismembering or while dismembering meat and feeding primate pets).

The nutritional habits of girls and boys with regard to NHP bushmeat seemed to be identical and comparable to their parents (approx. 60 % eat monkeys and 15 % chimpanzees). Adults consume NHP occasionally and buy mainly fresh or smoked meat on the local market. The main hunting methods reported were the application of gunpowder and setting of traps. People justified their abstinence of NHP consumption by traditional and personal reasons *(Table 11)*. 17.1 % of the study participants reported to be in touch with primate pets (mainly cercopithecus and cercocebus species) and 3.8 % had been injured by a monkey in the past (snakes, dogs and scorpions caused substantially more injuries) *(Table 11)*.

Different statistical models were calculated in order to analyze which factors influenced participants“to eat/dismember/cook or not to eat/dismember/cook monkey/chimpanzee”. The models with “*to hunt or not to hunt monkey/chimp*” as response failed to converge. Irrespective of the included predictors (*Birthplace: country* or *Ethnicity: Origin*) or of the answer (“to eat/dismember/cook or not to eat/dismember/cook monkey/chimp””), the GLMM were highly significant ($p<<0.01$) and explained 16-48 % of the variance as indicated by the conditional R2. Specifically, the model revealed the clear effect of sex, birth country and ethnicity on NHP bushmeat contact. For example men ate monkey significantly more often than women ($z=3.38$, $p<<0.01$) and women, in return, cook monkey significantly more often than men ($z=-5.94$, $p<<0.01$). Participants born in CI ate monkey significantly more often than people born in BF ($z=9.6$, $p<<0.01$). The same applies to consumption of chimpanzee ($z=3.411$, $p<<0.01$).The overall effect of age group was not significant. However participants from age group 1 (1-20 years) ate significantly less monkeys than age group 2 (21-40 years) ($z=2.14$, $p=0.03$) and age group 3 (41-60 years) ($z=2.1$, $p=0.04$). There was no significant difference between the other age groups.The different GLMMs including *Ethnicity: Origin* were more significant and revealed a higher conditional R2 than the GLMMs including *Birthplace: country*. For instance, one can quote the models analyzing the factors influencing participants to eat or not to eat monkeys: when *Ethnicity: Origin* was included as predictor, the GLMM was highly significant (likelihood ratio test: df=5, $p<<0.01$) and explained 48 % of the variance (R^2marginal= 0.45, R^2conditional= 0.48); by contrast when *Birthplace: country* was included as predictor, the GLMM was still highly significant (likelihood ratio test: df=5, $p<<0.01$), but explained only 39 % of the variance (R^2marginal= 0.36, R^2conditional= 0.39).

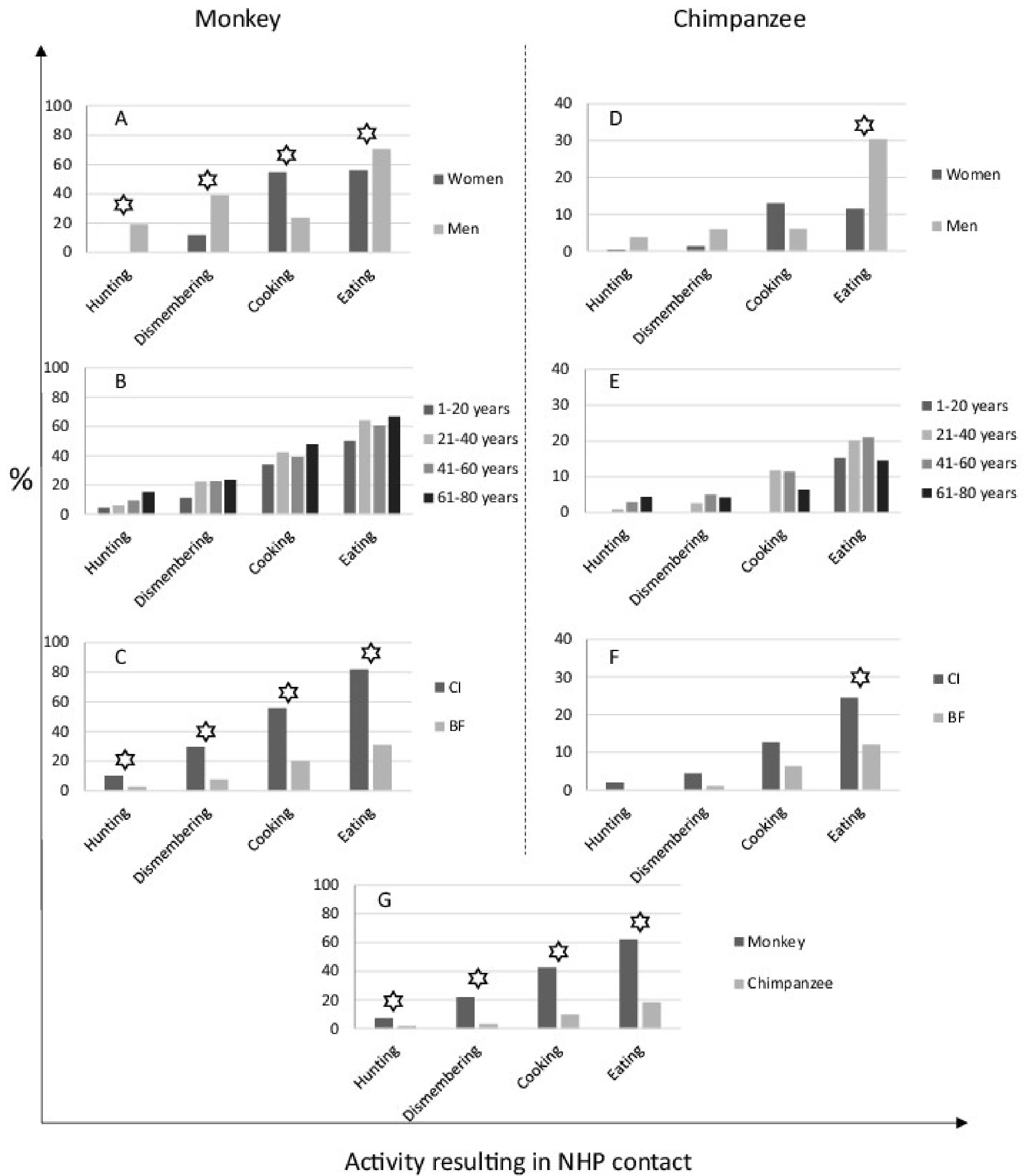


Figure 6. Frequency of exposure to non-human primate (NHP) bushmeat from monkey (A, B, C) and chimpanzee (D, E, F) of inhabitants of the *Taï* region, Western Côte d'Ivoire.

A, D: Comparison between men and women. B, E: Comparison of age groups. C, F: Comparing participants born in Côte d'Ivoire (CI) and Burkina Faso (BF). As results for country of birth and ethnic origin are very similar, the latter are only shown in Table 1. asterisk, difference statistically significant as revealed by General Linear Mixed Model (GLMM) analysis (all P values < 0.001).

		Sample size	Monkey				Chimpanzee				NHP pet
			Hunt	Dismember	Cook	Eat	Hunt	Dismember	Cook	Eat	Contact
Sex	Women	305	0.0	11.7	54.8	56.1	0.3	1.4	13.0	11.6	13.4
	Men	198	19.1	39.1	23.4	70.7	3.8	5.9	6.0	30.3	22.3
Age Group	1: <20years	46	4.5	11.1	34.1	50.0	0.0	0.0	0.0	15.2	13.3
	2: 21-40years	246	6.0	22.3	42.3	63.8	0.9	2.6	11.8	20.2	19.1
	3: 41-60years	147	9.2	22.5	39.2	60.5	2.9	5.1	11.5	21.1	16.2
	4: 61-80years	48	15.2	23.4	47.8	66.7	4.3	4.3	6.4	14.6	16.7
Country of birth	CI	263	10.3	29.5	55.8	81.7	2.0	4.4	12.7	24.5	27.6
	BF	190	2.8	7.8	20.1	31.1	0.0	1.1	6.3	12.1	4.9
	other	23	8.7	21.7	52.2	69.6	4.5	0.0	9.5	13.0	0.00
Ethnic origin	CI	238	11.5	34.2	62.0	90.3	3.1	5.3	13.8	25.4	28.9
	BF	245	3.1	7.7	22.3	33.9	0.0	0.9	6.6	12.3	5.4
	other	10	12.5	40.0	40.0	70.0	0.0	0.0	12.5	30.0	33.3
Sampling village	Daobly	92	2.4	18.4	25.3	45.7	1.2	0.0	3.7	13.0	25.3
	Ponan	81	3.3	10.0	34.4	45.3	1.2	4.8	15.7	21.1	15.2
	Gouleako	69	8.7	17.4	36.2	60.9	1.5	2.9	11.9	20.3	13.00
	Pauleoula	51	8.3	26.7	42.4	68.3	0.0	1.7	5.1	10.0	10.4
	Gahably	60	13.7	29.5	60.3	77.8	2.7	5.3	16.4	27.5	15.00
	Zaipobly	95	5.5	22.2	64.8	71.4	1.8	3.6	9.3	12.7	10.7
	Keibly	56	16.0	31.4	41.2	80.4	4.0	4.0	8.2	29.4	27.5
		Mean	7.7	21.3	42.2	61.9	1.7	3.2	10.2	19.2	17.2

Table 11. Activities resulting in contact with non-human primate (NHP) bushmeat. Listed are proportions of participants (in %) reporting to have been exposed to NHP, separately for contact to monkeys and chimpanzees, respectively.

4.2 AdV in humans from 4 Sub-Saharan countries: prevalence, phylogeny and pathogenesis (Pauly *et al.* 2014)

HAdV D generic nested PCR (HEX-HAdVD PCR) and sequencing revealed a HAdV D prevalence of 66 % (95%CI 56-76 %) in CI, 48 % (95%CI 38-58 %) in DRC, 28 % (95%CI 13-51 %) in CAR (adults only), and 65 % (95%CI 53-75 %) in UG (adults only). The prevalence in CI was significantly higher than in DRC ($P < 0.01$). When comparing the adult populations in all four countries, the prevalence in CI and UG was significantly higher than in DRC and CAR ($p < 0.05$). In CI and DRC, 100 % and 68 % of the younger children and 71 % and 5 0% of the older children and adolescents were HAdV D positive *(Table 12)* and the proportion of infected people decreased further to 64 % and 39 % in the adults in CI and DRC, respectively. Overall there was a significant decrease in the proportion of infected individuals with increasing age group (regression coefficient -0.6, $p < 0.05$). Gender had no significant effect on infection status ($p > 0.100$). The prevalence per village ranged from 45-100 % (data for individual villages not shown) and there was overall no significant difference between the villages ($p > 0.05$) (mean n/village = 16.7, range 2-38). The logistic regression model explained 11 % of the variance in the dataset (pseudo R-square = 0.1064).

To investigate the pathogenicity of HAdV D, we tested for a correlation between HAdV D shedding and clinical symptoms. Overall, 57 % of the study participants reported at least one clinical symptom. There was no correlation between infection status and individual clinical signs, or between infection status and poor health status, i.e. individuals that reported symptoms ($p > 0.05$) *(Table 12)*.

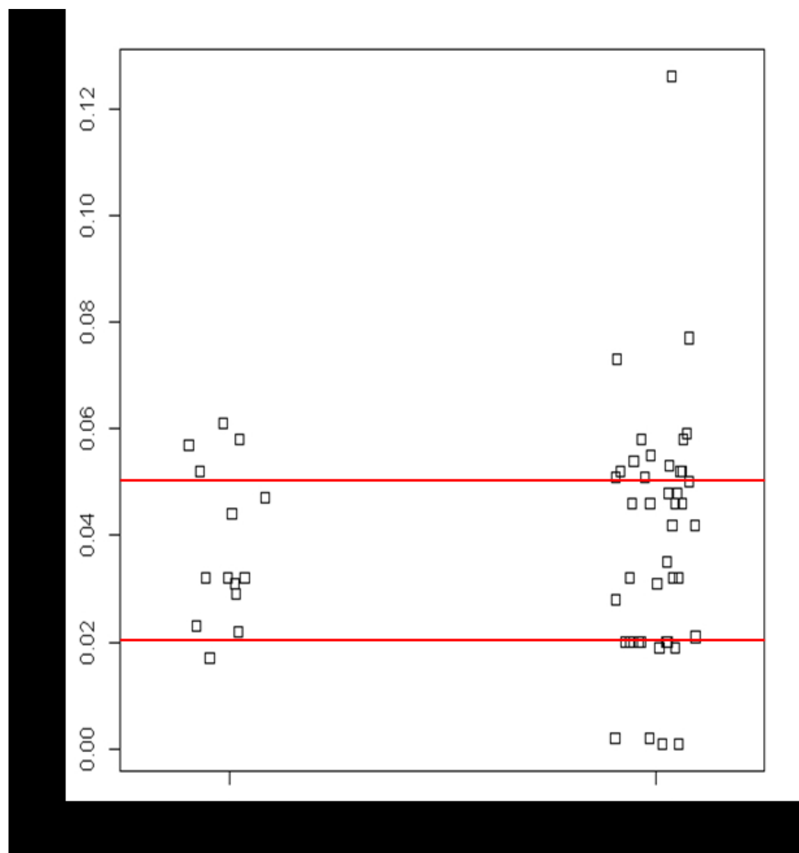


Figure 1. Comparison of observed minimum genetic distances.

In this strip-chart, observed minimum genetic distances (minGD) of human adenovirus species D (HAdV D) types are plotted. Left panel: minGD was determined for the 13 HAdV D sequences of this study in relation to 43 HAdV D types from Genbank. Right panel: minGD was determined for every HAdV D type from Genbank in relation to the other 42 Genbank types. The red lines indicate the first quartile (25 %) at 0.02 and the third quartile (75 %) at 0.05 of the minGD values from the 43 Genbank types. The interquartile range (0.05-0.02) gives the range of the middle 50 % of the observed minGD values of the Genbank types.

	Number of analysed individuals	% analysed individuals	Number of HAdV D positive individuals	% HAdV D positive individuals
Côte d`Ivoire				
Study group	95	100	63	66
0-5 years	4	4	4	100
6-19 years	7	7	5	71
20-77 years	84	88	54	64
male	42	44	30	71
female	53	56	34	63
asymptomatic	45	47	15	33
symptomatic	50	53	35	70
abdominal pain	21	22	14	67
diarrhea	3	3	3	100
nausea	1	1	1	100
Ocular disease	6	6	3	50
fever	10	11	9	90
Respiratory disease	8	8	6	75
headache	19	20	12	63
Democratic Republic of the Congo				
Study group	105	100	50	48
0-5 years	19	18	13	68
6-19 years	30	29	15	50
20-78 years	56	53	22	39
male	51	49	27	53
female	54	51	23	43
asymptomatic	41	39	36	88
symptomatic	64	61	27	42
abdominal pain	46	44	18	39
diarrhea	4	4	0	0
nausea	1	1	0	0
Ocular disease	0	0	0	0
fever	9	9	3	33
Respiratory disease	18	17	8	44
headache	14	13	6	43

Table 12. Human AdV species D (HAdV D) detection and clinical symptoms

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We finally analyzed if the sequences of the current study were derived from novel HAdV D types. For this purpose, we attempted to amplify a 4.8 kb fragment spanning the genes pV-hexon gene block

from 35 randomly chosen, HAdV D positive samples from CI, DRC, CAR and UG. Fragments were obtained from 14 samples after 2nd or 3rd round of Long-Distance PCR and completely sequenced. Thirteen different HAdV D sequences were obtained. Since in 2 samples from UG close to identical sequences were identified, only one of them (Hu4555_UG) was included in further analysis. BLAST analysis of the hyper-variable Loop 2 region from the 13 sequences revealed a pairwise identity of 86.4%-100% to known HAdV D types (HAdV D types 9, 13, 15, 17, 23, 25, 27, 29, 30, 32 47, 48, 56, 65, 67). We then compared the minimum genetic distances (minGD) of the pV-hexon sequences from 43 known HAdV D types with those of the 13 types identified in this study. First, we determined minGD between the 43 recognized types *(3.7 Phylogenetic analysis "Analysis of genetic distances of HAdV D detected in humans"*) to estimate the range of intertypic minGD. 50% of all values lay between 0.02 and 0.05 nucleotide substitution per site (*Figure 7*). Then, we determined minGD between the 13 sequences generated in this study and any recognized type: here 84.6% of all values lay between 0.02 and 0.05 nucleotide substitutions per site (*Figure 7; Table 13*). All 13 unique sequences identified in this study exhibited minGD >0.02; 5 sequences even exhibited a minGD >0.04, which outperformed >50% of intertypic minGD (*Figure 7 and Table 13*). Comparable results were obtained by using estimated instead of observed minGD (*Table 13*) and by analyzing only the region containing the hyper-variable hexon loops (Loops 1 and 2). These loops represent the major target for antibodies, are involved in immune escape, are particularly prone to recombination events and are among the target sequences for the characterization of HAdV types. Although more sequence information would be desirable, our data already point at 5 of 13 unique sequences likely representing novel types (under the conservative assumption that intertypic minGD > intratypic minGD in at least 50% cases). Recombination analysis of our data confirmed the tendency of HAdV D to recombine in the hyper-variable loop region of the hexon gene (data not shown).

Sample	Observed genetic distances						Estimated genetic distances					
	pVII-hexon gene			hypervariable loops			pVII-hexon gene			hypervariable loops		
	minGD study	n minGD Genbank < minGD study	% minGD Genbank < minGD study	minGD study	n minGD Genbank < minGD study	% minGD Genbank < minGD study	minGD study	n minGD Genbank < minGD study	% minGD Genbank < minGD study	minGD study	n minGD Genbank < minGD study	% minGD Genbank < minGD study
Hu4751_CI	0.02	4	9.30	0.00	6	13.95	0.02	4	9.30	0.00	0	0.00
Hu4806_CI	0.02	13	30.23	0.03	11	25.58	0.02	10	23.26	0.02	11	25.58
Hu4719_CI	0.02	13	30.23	0.04	13	30.23	0.02	10	23.26	0.04	11	25.58
Hu4787_CI	0.03	15	34.88	0.06	18	41.86	0.03	13	30.23	0.07	13	30.23
Hu4152_DRC	0.03	16	37.21	0.04	13	30.23	0.03	13	30.23	0.06	13	30.23
Hu4813_CI	0.03	16	37.21	0.02	10	23.26	0.03	13	30.23	0.01	11	25.58
Hu4882_CI	0.03	16	37.21	0.07	18	41.86	0.04	17	39.53	0.11	19	44.19
Hu4746_CI	0.03	16	37.21	0.07	18	41.86	0.03	16	37.21	0.25	22	51.16
Hu4109_DRC	0.04	22	51.16	0.12	20	46.51	0.05	22	51.16	0.26	22	51.16
Hu4108_DRC	0.05	26	60.47	0.11	20	46.51	0.05	22	51.16	0.13	20	46.51
Hu4564_UG	0.05	31	72.09	0.12	20	46.51	0.06	29	67.44	0.29	22	51.16
Hu4214_DRC	0.06	37	86.05	0.17	33	76.74	0.07	34	79.07	0.67	35	81.40
Hu4555_UG	0.06	37	86.05	0.16	26	60.47	0.07	37	86.05	0.50	31	72.09

Table 13. Comparison of the minimum genetic distance values of HAdV D type

4.3 AdV in domestic and wild mammals from rural Côte d`Ivoire: prevalence, phylogeny and pathogenesis (Pauly *et al.* 2015)

The aim of this part of the study was to detect AdV shed by domestic animals in 8 villages situated close to the Taï National park with PCR and sequencing. The diversity of AdV genotypes circulating in the study region was assessed, their phylogenetic relationships determined and probable recombination events identified. 213 rectum swabs of domestic mammals and 17 intestine samples of rodents were tested with two generic nested PCRs, targeting the well conserved hexon (Animal-HEX PCR) and polymerase gene (DPOL PCR) of mastadenoviruses. The tested samples were a random subset of the samples collected of various mammal species in rural Taï region in Western Côte d`Ivoire in 2012.

PCR and sequencing: prevalence and diversity of the detected AdV. We obtained an average AdV prevalence of 3.9 % (8/208; 95% CI 2-7.4%) with the DPOL PCR and of 21.7 % (50/230; 95% CI 16.6-27.6%) in domestic animals with the Animal-HEX PCR. As the Animal-HEX PCR showed the highest sensitivity and as only the ruminants were tested with the DPOL-PanAdV PCR, only the results of the Animal-HEX PCR system were considered for the statistical analysis.

More specifically, AdV was detected in 28 % (16/58) of dogs, 18 % (9/50) of sheep, 17 % (10/60) of goat, 7 % (1/14) of cows, 38 % (9/24) of pigs, 24% (4/17) of rats and 14 % (1/7) of monkeys. Thus, the highest prevalence of AdV shedding was determined for pigs (*Figure 8)*. Among the detected AdV types were caprine (n=4, 8%), porcine (n=6, 12%), simian (n=2, 4%), murine (n=4, 8%) and ovine (n=14, 28%), but also different human types (n=17, 34%). For 2 AdV positive samples, species identification was not possible as the chromatogram was of bad quality and hence the sequence too short for accurate analysis.

We detected caprine and ovine AdV from goats and sheep, and a porcine AdV from a cow. In rectum swabs of pigs porcine and human AdV were detected. Rodents shed murine AdV, and dogs ovine and human AdV. No canine AdV could be identified among the different types detected in dogs and no bovine AdV were detected in ruminants. Human AdV were also detected from rectum swabs of pigs, goats and sheep. AdV were shed by animals in every village tested, but the highest prevalence of AdV shedding was found in Gouleako (42.9%, 12/28), closely followed by Pauleoula (34.4%, 11/32). The lowest prevalence was found in Zaipobly, where only 9.5 % (2/21) shed AdV *(Figrue 13).* Besides the rectum swabs, blood samples of animals which were positive for human AdV were tested with the Animal-HEX PCR and the HEX-HAdVD PCR. All blood samples were negative (data not shown).

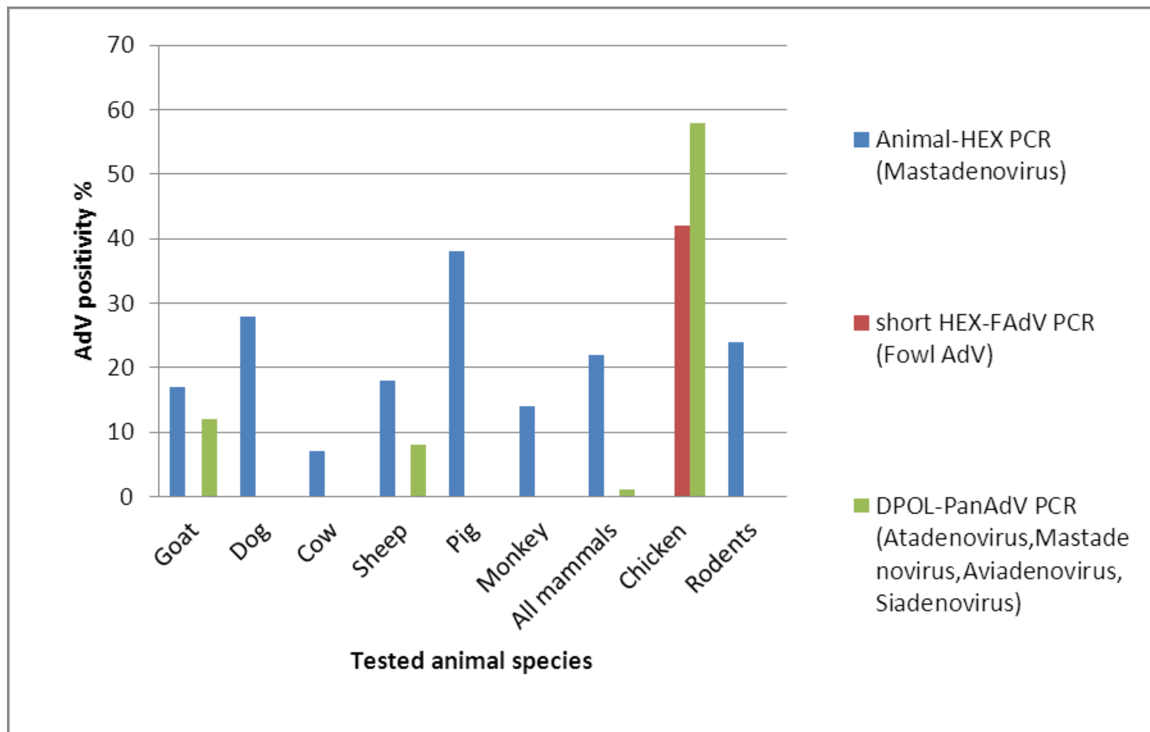


Figure 8. AdV positivity in domestic animals

This bar graph represents the percentage of AdV positivitiy of every animal species tested (goat, dog, cow, sheep, pig, monkey and chickens). All mammals were first tested with the Animal-HEX PCR system which targets the partial hexon gene of mastadenoviruses and all chickens samples were first tested with the short HEX-FAdV PCR system, which targets the partial hexon gene of Fowl adenoviruses (*Results 4.4 AdV in poultry AdV in poultry from rural Côte d`Ivoire: prevalence and phylogeny)*. Subsequently all ruminants (sheep, cow and goat) and the chickens were tested with the DPOL-PanAdV PCR, which targets the partial polymerase gene of AdV from nearly every genus (*Atadenovirus*, *Mastadenovirus*, *Aviadenovirus* and *Siadenovirus*).

Phylogenetic relation and species/type assignment of the detected AdV. The sequences obtained with the Animal-HEX PCR primers were too short and too well conserved to permit definite type identification and phylogenetic analysis of the detected AdV. In order to precisely characterize the detected AdV, a long-distance PCR was applied on all the positive animal samples (LD-HEX-MastAdV PCR). However, in spite of numerous trials, the amplification of the complete hexon gene was only successful for a small proportion of the previously detected AdV (28.8 %, 15/52). In total, 15 AdV sequences were obtained: from 1 sheep, 3 goats, 4 pigs, 1 monkey, 1 rat and 5 dogs. The sequences were assigned to the following AdV species: ovine AdV-5 (n=1), caprine AdV-2 (n=2), porcine AdV-3 (n=3), murine AdV-2 (n=1), HAdV-C2 (n=1), HAdV-D36,-D49, -D67, -D25, -D32 (each n=1) and HAdV-F41 (n=1). Blast research revealed for the ovine AdV 97 % pairwise identity to ovine AdV 5 (Genbank accession N°:DQ630758), for the caprine AdV 90.4 % pairwise identity to the caprine AdV-2 (Genbank accession N°DQ630760), for the murine AdV 90 % pairwise identity to the murine AdV-2 (Genbank accession N° HM049560) and for the porcine AdV 90.2-91.5 % pairwise identity to porcine AdV-3 (Genbank accession N°AJ237815). The pairwise identities mentioned are based on the nucleotide sequence.

For the simian AdV detected, no species assignment was feasible as the pairwise observed genetic difference of the amino acid sequence to every known simian AdV was 12.5 %, thus more than the minimum 5-15 % of amino acid sequence difference required for species demarcation (Harrach *et al.*

2011). This might be strong evidence that this simian AdV might not only be a new type, but the first strain of a novel species.

Most of the human AdV sequences detected in the animal rectum swabs were highly similar to already published HAdV types. Particularly the nucleotide sequences of HAdV C and F detected in dogs were >99 % similar to type HAdV-C2 (Genbank Accession N°JX173081) and HAdV-F41 (Genbank Accession N°AB610524). The HAdV D strains differed a bit more from the known types and the nucleotide sequence shared on maximum 98 % pairwise identity (HAdV-D36: *94 % pairwise identity; Genbank accession N°GQ384080;* HAdV-D49: *95.7 % pairwise identity; Genbank accession N°DQ393829;* HAdV-D67: *98.7 % pairwise identity; Genbank accession N° KF268206;* HAdV-D49: *96.2 % pairwise identity; Genbank accession N°JF799911;* HAdV-D32: *97.6 % pairwise identity; Genbank accession N° DQ149629)*. The pairwise identities mentioned are based on the nucleotide sequence.

Observed and estimated minimum genetic distances (minGD) of the HAdV D detected from human and animals in this study were compared to those of recognized HAdV D types from the Genbank with a similar approach as described above under "*AdV in humans from 4 Sub-Saharan countries: prevalence, phylogeny and pathogenicity*". This analysis revealed that some of the "animal" (and of the human) HAdV D might be novel HAdV D types *(Figure 9)*. None of the animal study sequences was 100 % identical to a Genbank sequence or to the HAdV D detected in the human study participants. The HAdV-D detected in animal rectum swab were more closely related to recognized Genbank sequences than to the HAdV-D detected in humans from the study region.

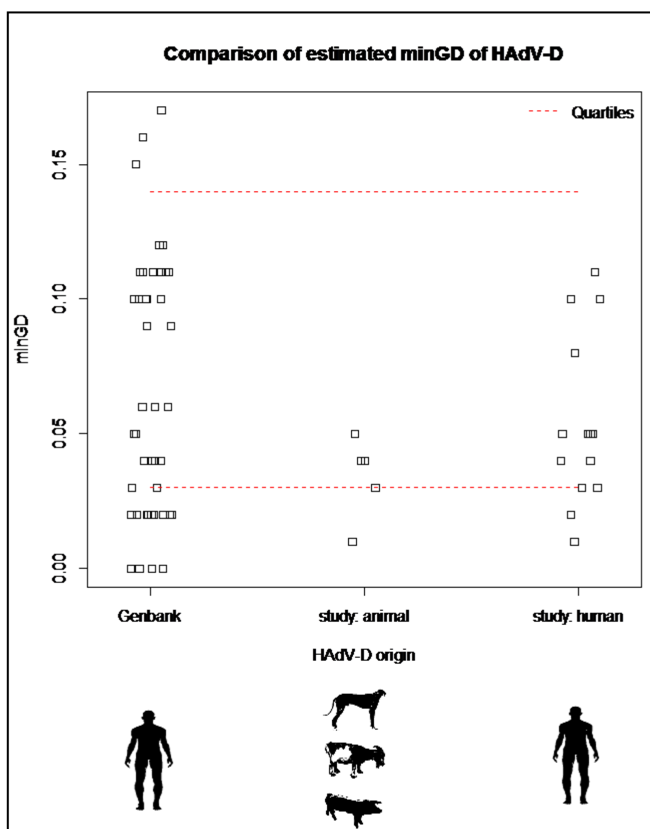


Figure 2. Comparison of estimated minimum genetic distance of HAdV D detected in this study from animals and humans with recognized Genbank sequences

In this strip-chart, estimated minimum genetic distances (minGD) of human adenovirus species D (HAdV D) types are plotted.
Left panel: minGD was determined for every HAdV D type from Genbank in relation to the other 42 Genbank types.
Right panel: minGD was determined for the 13 HAdV D sequences of this study in relation to 43 HAdV D types from Genbank.
Mid panel: minGD was determined for the 6 HAdV D sequences of this study in relation to 43 HAdV D types from Genbank.
The red lines indicate the first quartile (25 %) at 0.02 and the third quartile (75 %) at 0.11 of the minGD values from the 43 Genbank types. The interquartile range (0.02-0.11) gives the range of the middle 50 % of the observed minGD values of the Genbank types.

To further characterize the phylogenetic relation of the detected study sequences to known AdV types, phylogenetic trees were constructed with both, the maximum likelihood (ML) and the Bayesian approach. The murine AdV was taken as outgroup of the ML analysis. Phylogenetic analyses were based on an alignment of 1856 bp comprising 39 haplotypes of the study sequences and several strains from every recognized mastadenovirus species. As both consensus trees resulted in a similar, well supported, topolopy, only the ultrametric Bayesian tree is shown. Only the significant Bayesian posterior probabilities (pp>0.75) are depicted. The tree being a chronogram, the branches represent the evolutionary nucleotide changes over time.The first diverging event led to two well supported lineages: one comprises all the human and simian taxa, the other all the animal taxa. The murine AdV species clade formed the outroup. Several monophyletic sister clades were apparent, which represent the different recognized human and animal AdV species. Most of the study sequences clustered within these sister clades, regrouping known animal or human AdV. Moreover the clustering of the study sequences with the recognized AdV was supported by high pp values (pp>0.75). Thus species assignment could be made based on the tree topology. However, the study sequence MTAI277 did not clearly cluster with any monophyletic AdV species. The simian AdV detected in a mona monkey *(Cercopithecus mona*) in Taï village (MTAI277) seemed to be related to another simian AdV (SAdV-18, Genbank Accession N°FJ025931) and to the HAdV-F41 (Genbank Accesion N°AB610527) *(Figure 10)*.

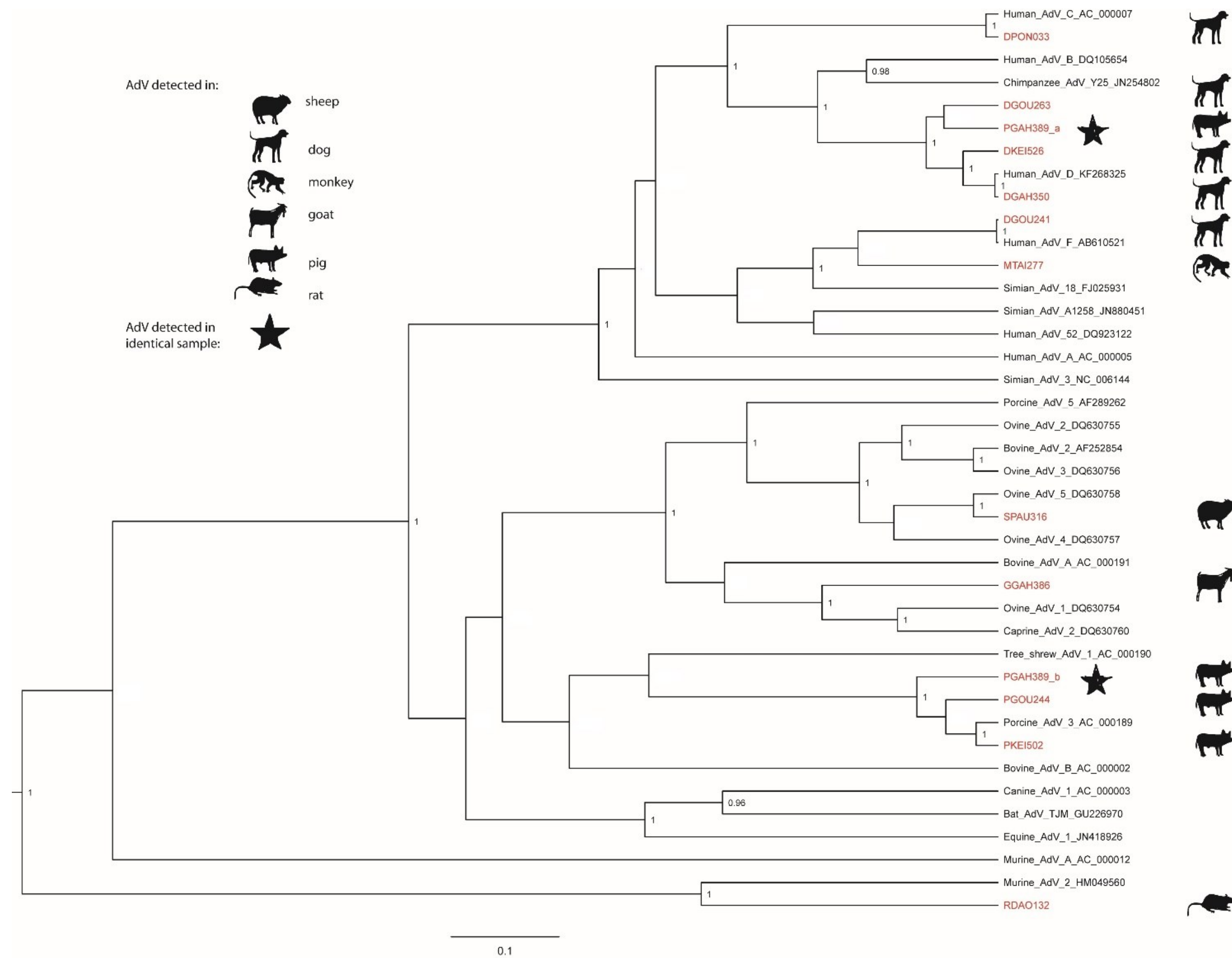


Figure 10. Phylogenetic tree of Mastadenovirus

Bayesian analysis of an 1856 bp long alignment of nearly complete hexon gene sequences, comprising at least one reference strain of every mastadenovirus species. The alignment comprised AdV sequences identified in this study and recognized reference strains from Genbank. A MCMC chain of chainLength 2000000 was run with the substitution model GTR+I+G, under a relaxed lognormal molecular clock and the prior assumption that the population size has remained constant throughout the time spanned by the genealogy. The reference strains are represented by host name, type and Genbank accession number. The study sequences are in red and the animal host can be retrieved from the first letter (P=pig, G=goat, S=sheep, M=monkey, D=dog, R=rodent). Adenovirus is abbreviated to AdV. Tree topology was tested by posterior probability and only the well supported values are shown (pp>0.75). The tree being a chronogram, the branches represent the evolutionary nucleotide changes over time.The tree was rooted using murine adenovirus A and B. The coloured ellipses represent the different host from which the AdV was detected. The two AdV marked by a red star were detected from the same sample (PGAH389).

Recombination analyses of the alignment comprising the sequences obtained with the LD-HEX-MastAdV PCR were performed to confirm this suspicion and to identify other potential recombination events, likely parental strains of recombinants and recombination hotspots. The program detected several recombination events involving study sequences. Similar to the human AdV (Crawford-Miksza *et al.* 1996), the loop regions of hexon gene were identified as potential recombination hotspot. However, when the AdV sequence detected from MTAI277 was compared to other recognized simian AdV {dataset of (Roy *et al.* 2012)}, it was not among the proposed recombinants. Of particular interest were also the two AdV sequences obtained from the pig PGAH389; this piglet from Gahably shed not only HAdV D, but also porcine AdV-3. Potential recombination events involving both the HAdV D and the porcine AdV-3 were identified by the recombination program. However, the events were not well supported by all the recombination detection methods embedded in RDP4 (Martin *et al.* 2005, Martin *et al.* 2010).

To elucidate the phylogenetic relation of the SAdV sequence obtained from the mona monkey, MTAI277, phylogenetic analyses of an alignment comprising the MTAI277 SAdV sequence, other SAdV and closely related HAdV sequences were conducted with maximum likelihood and Bayesian inference. The selected SAdV were identical to those included in the phylogenetic analysis of AdV from monkeys (e.g. rhesus macaques) (Roy *et al.* 2012). Moreover a gorilla and a chimpanzee AdV closely related to the HAdV F were added in the analysis (Wevers *et al.* 2011). As both consensus trees resulted in a similar, well supported topolopy, only the ultrametric Bayesian tree is shown. The AdV types of each human and simian species formed distinct and well supported clades. The MTAI277 sequence stands on a well supported isolated branch of the Bayesian tree and is closely related to the SAdV-18 and the clade comprising the HAdV F isolates and the two AdV sequences detected in a gorilla and a chimpanzee (Wevers *et al.* 2011) *(Figure 11)*.

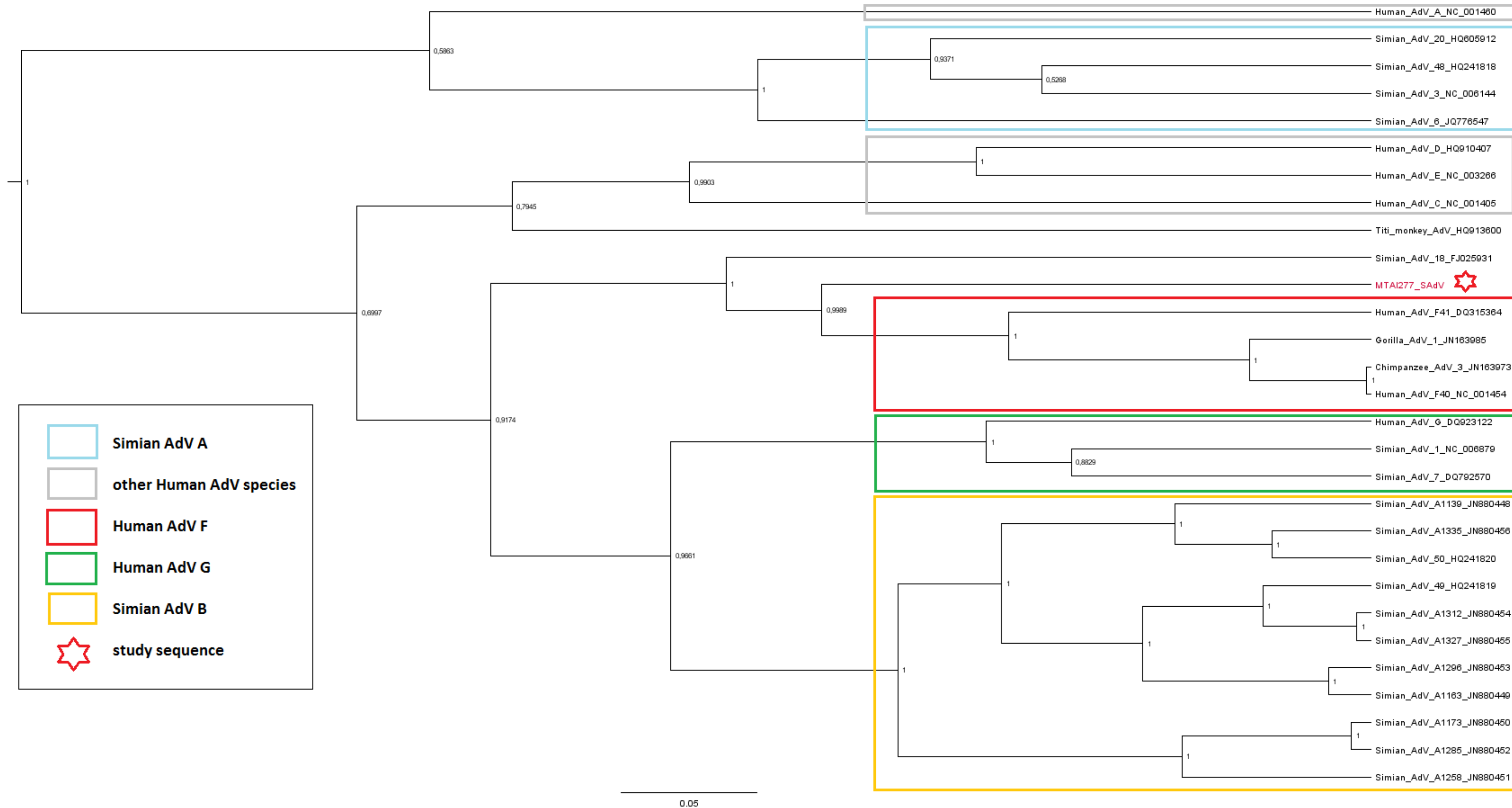


Figure 11. Phylogenetic tree of Simian AdV

Bayesian analysis of a 2400 bp long alignment of nearly complete hexon gene sequences, comprising mainly simian and closely related human adenovirus sequences. The alignment comprised AdV sequences identified in this study and recognized reference strains from Genbank. The selected simian AdV are identical to those included in the phylogenetic analysis of AdV from monkeys (e.g. rhesus macaques) (Roy et al, 2012). Moreover a gorilla and a chimpanzee AdV closely related to the human adenovirus F were added to the analysis (Wevers et al, 2011). A MCMC chain of chainLength 2000000 was run with the substitution model GTR+I+G, under a relaxed lognormal molecular clock and the prior assumption that the population size has remained constant throughout the time spanned by the genealogy.The reference strains are represented by host name, type and Genbank accession number. The study sequence is in red and was detected from a habituated mona monkey. Adenovirus is abbreviated to AdV. Tree topology was tested by posterior probability and only the well-supported values are shown (pp>0.75). The tree being a chronogram, the branches represent the evolutionary nucleotide changes over time. The tree was rooted using human adenovirus A. The coloured boxes represent the different recognized simian and human species

Phylogenetic analyses were also carried out with the sequences obtained with the DPOL-PanAdV PCR system. All ruminant (cow, sheep and goat) and chickens samples were tested with this PCR system, which targets the polymerase gene of potentially all known AdV genera (Wellehan *et al.* 2004). No atadenovirus and only few mastadenoviruses were detected in the ruminant samples, including HAdV D (n=2), porcine AdV (n=1) and bovine AdV (n=8). Low pairwise identity to the next closely related AdV sequence available in Genbank or bad quality of the chromatogram obviated definite species assignment of the human and porcine AdV. Blasting of the bovine AdV revealed 82 to 98 % sequence identity to bovine AdV2 (Genbank accession N° AF252854).This BLAST result largely differed from the result obtained for the AdV hexon gene sequences (Animal-HEX PCR), particulary concerning the detected bovine AdV. The findings obtained from the chicken survey are described under *"AdV in poultry from rural Côte d`Ivoire: prevalence and phylogeny"*. Although the length of the sequences obtained was only approx. 270 nucleotides, phylogenetic analyses with acceptable branch support were possible with Bayesian and maximum likelihood inference. Phylogenetic analyses were based on an alignment of 191 bp comprising 52 haplotypes of the study sequences and available polymerase sequences from each genus. As both consensus trees resulted in a similar, well supported, topolopy, only the ultrametric Bayesian tree with the thus far sole *Ichtadenovirus* (the Sturgeon AdV) (Kovács *et al.* 2003)as outgroup is shown. The AdV types of each genus formed 5 distinct well supported clades. With the genus *Ichtadenovirus* as outgroup, *Aviadenovirus* was the next most ancient clade and the lineages of *Atadenovirus, Siadenovirus* and *Mastadenovirus* diverged more recently. The majority of the study sequences clustered within the genera *Mastadenovirus* and *Aviadenovirus* and the only few siadenovirus taxa were detected in chickens. For definite resolution of the relationship of the study strains with the recognized Genbank strains, the sequences obtained were to short and their topology too weekly supported *(Figure 12).*

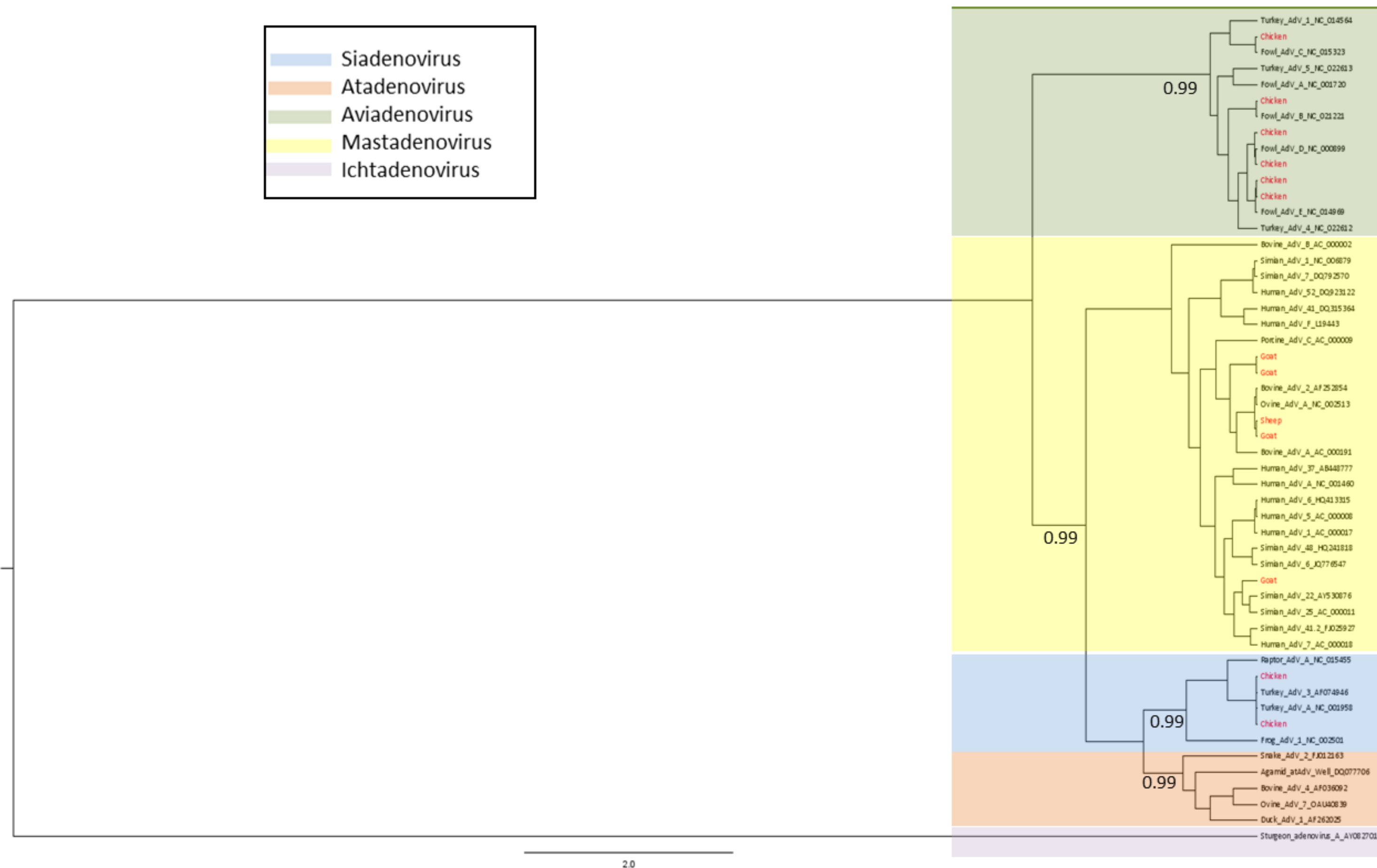


Figure 12. Phylogenetic tree of Adenoviridae

Bayesian analysis of an 199 bp long alignment of the partial polymerase gene (Wellehan *et al.* 2004), comprising the adenoviruse sequences from every genus. The alignment comprised AdV sequences identified in this study and recognized reference strains form Genbank. A MCMC chain of chain Length 2000000 was run with the substitution model HKY+G, relaxed lognormal molecular clock and the prior assumption that the population size has remained constant throughout the time spanned by the genealogy.The reference strains are represented by host name, type and Genbank accession number. The study sequences are in red and the host can be retrieved from the taxa name (Chickens, Goat and Sheep). Adenovirus is abbreviated to AdV. Tree topology was tested by posterior probability and only the well supported values for the genus classification are shown (>0.75). The tree being a chronogram, the branches represent the evolutionary nucleotide changes over time.The tree was rooted using white sturgeon adenovirus 1. The coloured boxes represent the different recognized AdV genera

Statistical analysis: factors influencing AdV shedding. Basic descriptive statistics revealed that a higher proportion of female (n=121, 60 %) as compared to male were tested (n=81, 40 %). Moreover the analyses showed that more adult (n=133, 64.6 %), than juvenile animals (n=73, 35.4 %) were screened for AdV and that the majority of the animals were in good coat (130/200, 65 %) and nutritional condition (160/203, 79 %). Moderate nutritional and coat condition were rarely observed *(Table 14)*. The good health status of most of the animals was also supported by the median and mean body temperature of approx. 38.8°C and the mean body temperature measured (the average body temperature of healthy mammals is 38-39°C). The high body temperature (maximum body temperature measured: 42.5°C) of some animals was partly stress- and weather-related (ambient temperature of approx. 25-40°C during sampling), but also bacterial or viral infections might have been the cause. On several occasions, symptoms of endometritis, mastitis, pneumonitis and gastroenteritis were observed *(Table 14)*.

animal species	n	n	Gender		Age		nutritional condition		coat condition		Body Temperature	
	sampled	tested	Female	Male	Adult	Juvenile	Good	Moderate	Good	Moderate	Mean (°C)	Median (°C)
Cow	17	14	9	5	8	6	12	2	12	2	38.7	38.8
Dog	90	58	25	31	42	15	34	21	24	31	38.6	38.7
Goat	120	60	40	18	41	18	52	5	48	8	39.2	39.1
Monkey	7	7	2	5	4	3	6	1	5	2	38	38
Pig	34	24	15	9	1	23	13	11	8	15	39.3	39.1
Sheep	103	50	30	13	37	8	43	3	33	12	39	39
Rodent	17	17	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Chickens	106	91	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

Table 14. Descriptive statistics of demographic data of the animal study participants from CI

For some animals the demographic data were incomplete

In order to analyse which factors influence AdV shedding, we analyzed the data using a Generalized Linear Mixed Model (GLMM) with binomial error structure and logit link function. The general idea of the model was that the categorical predictors (animal species, age and sex) and the random effect (sampling village) might influence AdV shedding. To establish the significance of the full model we used a likelihood ratio test, comparing its deviance with that of the null model comprising only the intercept. Overall, the full model was highly significant as compared to the null model (likelihood ratio test: R^2marginal= 0.15, R^2conditional= 0.18, df=4, p=0.03). Hence by including the village as random effect a better effect size could be obtained for the full model (R^2conditional= 0.18 compared to R^2marginal= 0.15). Specifically, the model revealed the clear effect of sex on AdV presence: female shed AdV significantly more often than male (z=-2.6, p= 0.01). There was no significant difference of AdV shedding between sheep and goat or sheep and dog. However the difference between dog and goat was significant (z=-1.9, p=0.05). Age had no obvious impact on AdV shedding (z=1.12, p=0.26). The second model, describing the association between health status (body temperature and nutritional condition) and AdV shedding, was not significant (p>0.05).

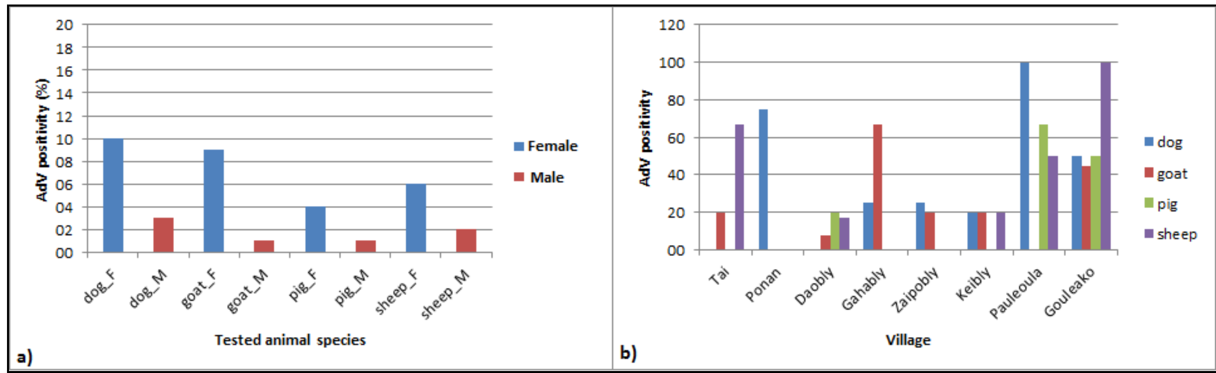


Figure 13. Effect of gender and village on AdV positivity.

a) The bar graph represents the proportion of females (F) and males (M) among the AdV positive animals of every animal species tested (goat, dog, sheep and pig). The AdV positivity is based on the result obtained by application of the Animal-HEX PCR system which targets the partial hexon gene of mastadenoviruses. As only few cows and monkeys were tested, we did not show those data.

b) The bar graph represents the percentage of AdV positive animals (goat, dog, sheep and pig) in every village (Taï, Ponan, Daobly, Gahably, Zaipobly, Keibly, Pauleoula and Gouleako). The AdV positivity is based on the result obtained by application of the Animal-HEX PCR system which targets the partial hexon gene of mastadenoviruses.

4.4 AdV in poultry from rural Côte d`Ivoire: prevalence and phylogeny (Pauly *et al.* 2015)

The aim of this part of the study was to detect AdV shed by domestic birds in 7 villages situated close to the Taï National park with PCR approach. No chicken samples from Gahably were available. While in Gouliako 27 chicken samples were collected and tested, the number of chickens decreased to only 3 in Taï village. The average amount of chickens tested per village was 11.4.The diversity of avian AdV genotypes circulating in the study region was assessed and their phylogenetic relationships determined. Avian AdV of the genus *Aviadenovirus*, but also of the genus Siadenovirus were detected in cloacal swabs collected from chickens (n=91). 3 generic nested PCRs were applied in the present study: two generic PCRs targeting the hexon gene of FAdV (short HEX-FAdV PCR and long HEX-FAdV PCR) (Meulemans *et al.* 2001) and a generic DPOL-PanAdV PCR targeting the polymerase gene of AdV belonging to different genera (*Aviadenovirus*, *Mastadenovirus*, *Siadenovirus* and *Atadenovirus*)(Wellehan *et al.* 2004).

FAdV PCR and Phylogenetic analysis: prevalence and diversity of circulating FAdV. The first screenings with the nested short HEX-FAdV PCR, 42.9 % of the chickens were FAdV positive (39/91; 95% CI 33-53 %). From 33 % (in Daobly) up to 74 % (in Gouliako) of the tested chickens were FAdV positive in each village. Since the primer target a very short hexon gene fragment, only FAdV species identification, but not type identification was made. FAdV of species B (n=10), D (n=14) and E (n=8) could be identified. Hence the most prevalent FAdV species was species D, which represented 35.9 % of the detected FAdV. Species D was followed by species B, which represented 25.6 % of the detected FAdV. No FAdV from species A and C were detected. In three villages (Ponan, Daobly and Pauleoula), FAdV species B, D and E were detected, while in Gouliako and Zaipobly only FAdV from species D and E were detected. In Keibly only FAdVD and in Taï only FAdVE appeared to ciculate *(Table 15)*. To determine the AdV type, 39 positive samples of this first screening were subsequently tested with generic FAdV primers established before and applied in many studies on FAdV prevalence (Meulemans *et al.* 2001). These primers, referred to as long HEX-FAdV PCR in the present study, target the conservative regions which flank the L1 loop and the resulting PCR product has a length of about 550 bp. From 71.8 % (28/39) of the tested samples FAdV sequences were obtained. 79 % of these sequences (22/28) presented a good chromatogram quality and phylogenetic and species delineation analyses were based on these sequences. The others (6/28) often presented noticeable baseline noise or double peaks, which might be due to co-infection with different FAdV types *(Table 15)*.

With the long HEX-FAdV PCR, FAdV of species B (n=5), C (n=1), D (n=10) and E (n=13) were identified. Hence, the most prevalent FAdV species was species E, which represented 46 % of the detected FAdV. No FAdV from species A was detected. In Daobly, all four FAdV species and in Ponan and Gouliako FAdV species B, D and E were detected. In Pauleoula only FAdV from species B and E, in Zaipobly and Keibly only species D and E were detected. The only FAdV species detected in Taï was assigned to species E. This unbalanced diversity among the village could be the result of the varying amount of chickens tested per village *(Table 15)*. In 36 % (10/28) of the cases, the FAdV detected with the long and short HEX-FAdV PCR system differed and thus the chickens might have been coinfected with different FAdV *(Table 15)*.

	Ponan		Tai		Daobly		Gouliako		Pauleoula		Zaipobly		Keibly		All villages	
PCR	**short HEX-FAdV**	**long HEX-FAdV**	**short HEX-FAdV**	**long HEX-FAdV**	**short HEX-FAdV**	**long HEX-FAdV**	**short HEX-FAdV**	**long HEX-FAdV**	**short HEX-FAdV**	**long HEX-FAdV**	**short HEX-FAdV**	**long HEX-FAdV**	**short HEX-FAdV**	**long HEX-FAdV**	**short HEX-FAdV**	**long HEX-FAdV**
FAdVA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FAdVB	4	2	0	0	5	1	0	1	1	1	0	0	0	0	10	5
FAdVC	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1
FAdVD	1	1	0	0	2	5	3	1	3	0	2	1	3	2	14	10
FAdVE	1	1	1	1	1	2	3	4	1	2	1	2	0	1	8	13
undefined FAdV	2	0	0	0	2	0	1	0	0	0	1	0	1	0	7	0
All FAdV	8	4	1	1	10	8	7	6	5	3	4	3	4	3	39	28
All tested	16	8	3	1	15	10	29	8	9	5	13	4	9	4	91	39

Table 15. FAdV positivity and diversity in chickens (n)

With the FAdV sequences obtained with the long HEX-FAdV PCR phylogenetic trees based on maximum likelihood and Bayesian inference were constructed. Phylogenetic analyses were based on an alignment of 471 bp comprising 57 haplotypes of the study sequences and several strains from every recognized FAdV serotype. Only the Bayesian tree is shown, and only significant Bayesian posterior probabilities (pp>0.75) are depicted. The tree being a chronogram, the branches represent the evolutionary nucleotide changes over time. 5 well supported monophyletic clusters, representing the 5 fowl AdV species, were identified. Every study sequence clustered within one of these clusters. Moreover the clustering of the study sequences with the recognized AdVs was supported by high pp values (pp>0.75) and thus species assignment based on the tree topology was feasible. The FAdV-5 strain TR22 formed a separate clade, which might stand for a sixth FAdV species. While most of the study sequences formed sister groups with recognized FAdV types, others were situated on separated branches and were thus only distantly related to recognized types. Moreover subclades within the species clades were apparent and well supported *(Figure 14)*.

The study sequences were compared to Genbank sequences of all the recognized FAdV serotypes and assigned to a certain species and genotype according to the demarcation criteria (nucleotide sequence identity of approx. 72 % for species and of 95 % fo type identification) proposed by Marek et al, 2010 (Marek *et al.* 2010). With these criteria, all detected sequences could be assigned to a recognized species and most to a recognized genotype. For some type identification was not feasible, the sequence identity to any recognized species being less than the proposed threshold value (95 %). This was the case for one FAdV from species B and for four FAdV sequences from species E *(Table 16)*. The most frequently identified FAdV were from species E and D. Only one strain of species C and five of species B could be obtained and no type from species A was detected *(Table 16).*

Study Sample	Maximal genetic identity	Species assigned	Type assigned
CPAU286	97.2	B	B1
CPON047	97.4	B	B1
CGOU224	97.4	B	B1
CPON040	99.8	B	B1
CDAO182	82.1	B	n.d.
CGOU223	93.9	E	n.d.
CDAO197	94.8	E	n.d.
CPAU281	87.8	E	n.d.
CZAI462	87.8	E	n.d.
CGOU266	99.3	E	E2
CGOU234	99.3	E	E2
CGOU267	99.3	E	E2
CPON002	98.7	E	E2
CPON012	97.8	E	E3
CZAI445	97.8	E	E3
CDAO107	99.6	D	D3
CKEI522	99.6	D	D3
CKEI519	97.4	D	D2
CZAI444	97.1	D	D2
CDAO164	99.6	D	D1
CDAO120	99.6	D	D1
CDAO110	98.2	C	C1

Table 16. Species and type identification of the FAdV by application of the criteria by Marek et al, 2010

The study sequences were compared to Genbank sequences of all the recognized FAdV serotypes to determine the Maximal genetic identity and assigned to a certain species and genotype according to the demarcation criteria (nucleotide sequence identity of approx. 72 % for species and of 95 % fo type identification) proposed by Marek et al, 2010 (Marek et al. 2010). n.d. means not determined, as found maximial genetic identity exceeded the threshold value for type identification.

Delineation analysis of FAdV species. In order to make statistically supported statements on the FAdV species circulating in the Taï region and to elucidate the evolution of FAdV species, delineation analyses were performed. The alignment used for phylogenetic analyses was analyzed with Bayesian MCMC and then delineation analyses were run applying the Generalized Mixed Yule Coalescent (GMYC) method. The single step method revealed to be significant (p-value=0.04) and the number of species clusters was estimated to be 12 (confidence interval: 3-16). In the resulting ultrametric tree, the proposed species clades were distinguished by differently coloured branches. The only recognized species, which was monophyletic and for which the delineation analysis confirmed the classification into a single separate species was FAdV A (AIC weight = 0.9). The other previously recognized FAdV species were divided into several subspecies. FAdV E was subdivided into at least 4 subspecies, but with a bad support (AIC weight<0.1). Similarly, FAdV B and C were subdivided into 3, and FAdV D into at least 4 subspecies with good support (AIC weight > 0.1). Furthermore, some of

the study sequences (e.g. CDAO110, CPON012, CDAO120) clustered in one of the defined (sub-) species with recognized FAdV types, while others would stand on a separate branch, identified as (sub-)species by the GMYC method (e.g. CDAO182, CGOU223, CZAI402). The GMYC methods hence proposed these strains to be sole types of a new (sub) species *(Figure 15)*. Consistent with the species subdivision proposed by Marek et al, 2010 (Marek *et al.* 2010), the identified subspecies could be named by adding a number to the species letter (e.g. FAdV-B1 to -B3). Moreover the analysis confirmed the hypothesis of previous studies (Meulemans *et al.* 2004, Marek *et al.* 2010) that the Japanese strain TR22 (FAdV-5) might be a member of a separate species (AIC weight <0.1).

PanAdV PCR and Phylogenetic analysis: prevalence and diversity of circulating avian AdV. In order to get a more complet picture ofavian AdV occurrence in the study region, all the chicken samples were tested with a generic DPOL-PanAdV PCR. Avian AdV were detected in 58.2 % (53/91; 95%CI 48-67.8 %) of the tested cloacal swabs from chickens. Among the detected avian AdV were mainly FAdV (90% of the detected avian AdV, 53 % of the tested chickens), but also siadenoviruses (10 % of the detected avian AdV, 5 % of the tested chickens). No AdV strain from genus Atadenovirus was detected. Similarly to the results with the PCR systems targeting the hexon gene (short HEX-FAdV PCR), FAdV from different FAdV species were detected. The majority of the FAdV detected belonged to species B (n=18, 34 %). Sequences of bird siadenoviruses were only obtained in three villages: Taï, Gouliako and Pauleoula. Phylogenetic analyses were performed with the obtained FAdV sequences as described under results section *4.4 AdV in domestic and wild mammals from rural Côte d`Ivoire: prevalence, phylogeny and pathogenicity (Figure 12)*. To further characterize the detected siadenovirus strains, a species-specific (potentially genus-specific) PCR system (SiAdV-HEX-PCR) was designed targeting a 538 bp long fragment of the Hexon gene of Turkey AdV-3 (and the other recognized siadenoviruses). The 4 sequences obtained were nearly identical and the BLAST analysis revealed up to 99 % pairwise identity with the already published Hemorrhagic enteritis Virus (Turkey AdV-3) (Genbank accession N° AF074946.1) (Pitcovski *et al.* 1998).

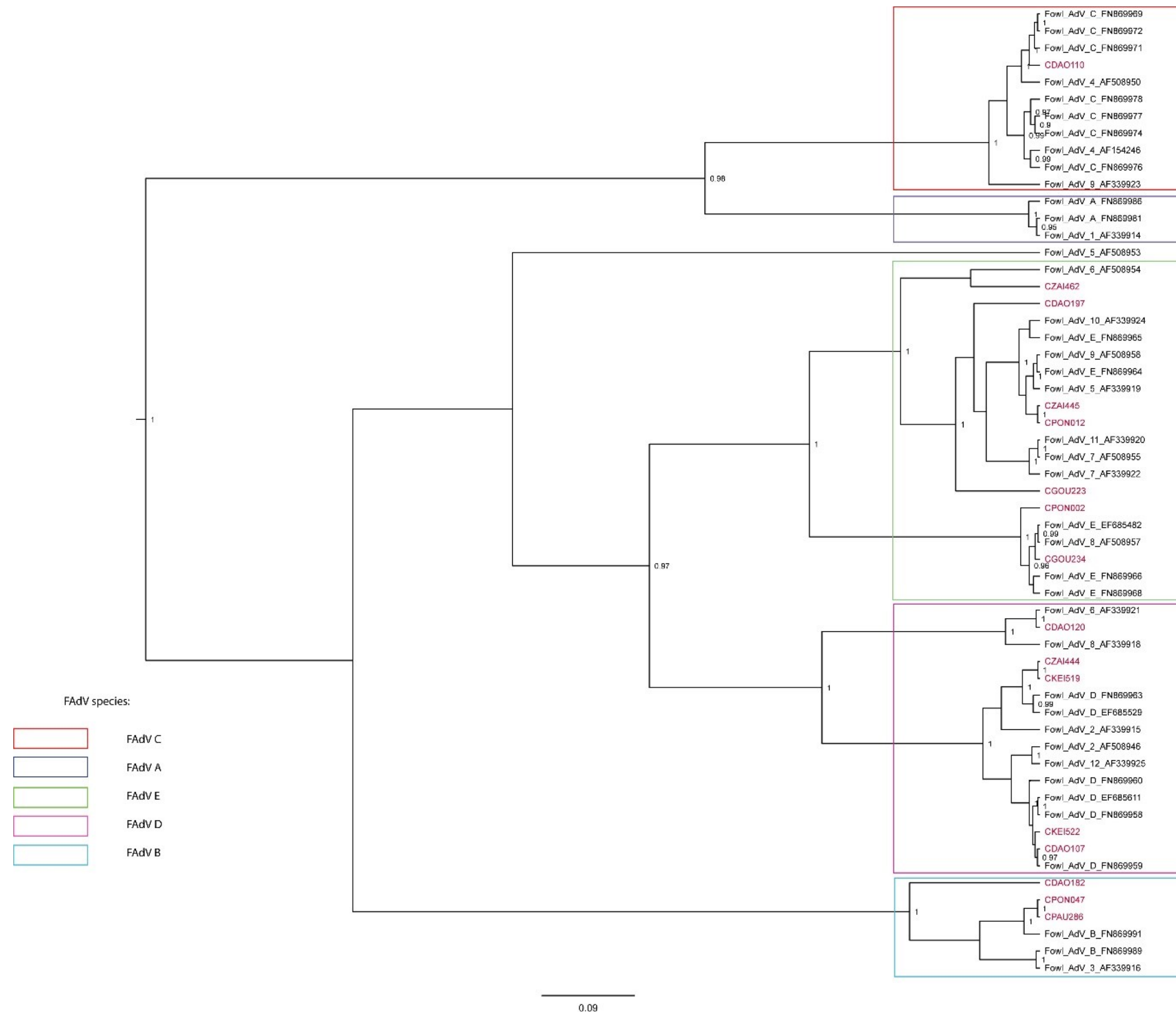


Figure 14. Phylogenetic analysis of FAdV

Bayesian analysis of a 471 bp long alignment of partial hexon gene sequences, comprising at least one reference strain of every Fowl adenovirus species and sequences identified in this study. A MCMC chain of chainLength 2000000 was run with the substitution model GTR+I+G, under a relaxed lognormal molecular clock and the prior assumption that the population size has remained constant throughout the time spanned by the genealogy. The reference strains are represented by host name, type and Genbank accession number. The study sequences are in red and were all detected in chicken cloacal swabs. Adenovirus is abbreviated to AdV. Tree topology was tested by posterior probability and only the well supported values are shown (pp>0.75). The tree being a chronogram, the branches represent the evolutionary nucleotide changes over time.The tree was rooted using the cluster comprising species fowl adenovirus A and C. The coloured boxes represent the different recognized fowl adenovirus species.

4.5 Zoonotic transmission of AdV in a rural region of Côte d`Ivoire (Pauly *et al.* 2015)

As we assumed that shedding of animal AdV by humans would represent a first indication that zoonotic transmission of animal AdV occurs, the investigation of the zoonotic potential of AdV was done by testing the human fecal samples for animal AdV. 2 different approaches were used to assure isolation of animal AdV even from samples with low copy numbers of animal virus and to simultaneously limit the amplification of the closely related and highly prevalent human AdV (e.g. HAdV D).

In a first approach, we tested all the human system with a PCR system, in which 2 primer types were concurrently applied: amplification primer and blocking primer (Blocking-HAdVD PCR). The idea was that these blocking primers would only prevent the amplification of the HAdV D, but would allow the amplification of the animal AdV. The PCR system was successfully tested before application on the study samples and appeared to provide reliable results concerning the zoonotic potential of AdV. Although some of the PCR products were of the expected length as visualized by gel electrophoresis, no animal AdV sequence could be obtained from the extracted gel bands.

In the second approach, all human samples were tested with primers specifically targeting animal AdV (FAdV and ruminant AdV) in order to control the results of the first method and to assure that the blocking primers would not block the amplification of the animal AdV leading to false-negative results. The primer pairs used for the specific amplification of FAdV were the same then those successfully applied on the poultry samples for the investigation of FAdV shedding. After ensuring that they did not amplify human AdV, the PCR system was directly applied on all the human fecal samples. No FAdV sequence could reproducibly be obtained from the human fecal samples.The primer pairs used for the specific amplification of ruminant AdV (ruminant-HEX PCR) were specifically designed based on ruminant sequences obtained with the Long-distance primer from animal samples (LD-HEX-MastAdV PCR). After ensuring that they did not amplify human AdV, the PCR system was directly applied on all the human fecal samples. No ruminant sequence could be obtained from the human fecal samples.

Thus, the zoonotic potential of AdV could not be demonstrated with any approach, neither with the PCR applying blocking primer nor with the PCR applying specific primers.

5 Discussion

The discussion section is divided into four parts. In the first three parts (5.1-5.3), the implications of the observed high prevalence and diversity of AdV shedding by humans and animals are discussed. Moreover the results are compared to similar studies from other regions of the world, possible virus transmission modes are highlighted and factors affecting AdV shedding are stressed. The last section (5.4) focuses on risk factors for cross-species disease transmission in the study region resulting from the environmental and political condition and from the local nutritional habits. Moreover this section elicites the role of the possibly consequential cross-species transmission of AdV for virus spread and for human and animal health.

5.1 HAdV-D in humans from rural Côte d`Ivoire (Pauly *et al.* 2014)

In this study, we estimated a relatively high prevalence of HAdV D in several rural study populations in Sub-Saharan countries (66 % in CI, 48 % in DRC, 28 % in CAR, and 65 % in UG). The overall prevalence in CI was significantly higher than in DRC, which was also reflected by the fact that the proportion of HAdV D-positive individuals across all age classes was higher in CI compared to DRC (*Table 12)*. UG also had a particularly high prevalence when comparing the adult populations only. Since we were able to explain only 11 % of the variance in the dataset with the statistical model, there are likely to be many factors that influence the difference in HAdV D occurrence which have not been included in this study. Considering the fecal-oral nature of HAdV transmission, such factors may be sources of local water supply and hygiene measures including toilet facilities. Also, nutrition and the local occurrence of HIV and other infections might play a role in the susceptibility to, and shedding of, HAdV D. HIV prevalence was not determined for the populations in the present study, but it is tempting to speculate that the high prevalence in CI is partly a result of the relatively high HIV-1 prevalence in the Taï area (7.2 %) (Ayouba *et al.* 2013).

Various sampling strategies and detection methods limited the feasibility of direct comparison of our cross-sectional study to other studies (*Supplementary Table 1)*. Study participants were within limited age groups, showed specific symptoms and/or different life styles. In contrast to other studies, we applied a generic nested PCR using non-degenerate primers that target the hexon gene of all known HAdV D types and sequenced all positive samples. Most primers implemented in other studies were degenerate and targeted several HAdV species. If HAdV species were confirmed by sequencing, this was only performed for a selection of the PCR products (*Supplementary Table 1*). This might have resulted in a considerable underestimation of the HAdV D prevalence

Our study included participants of all age groups ranging from young children, and older children/adolescents to adults. We observed that younger individuals shed HADV D significantly more frequently than adults, which shows on one hand that exposure to this infection likely occurs early in life and on the other hand that adults might develop immunity leading to a reduction in HAdV D shedding. It is not clear, whether this high prevalence can be explained by a general high sensitivity of children to any infection, or by a more likely ingestion of contaminated material in young children compared to adults. Although mothers should in theory be at higher risk of getting infection through baby care, no difference between men and women regarding HAdV D shedding was observed. This could indicate that the majority of infections occur via generally available sources in the villages (*Table 12)*.

It has been shown that some HAdV D types induce specific symptoms (Kaneko *et al.* 2009, Walsh *et al.* 2009, Arnold *et al.* 2010, Matsushima *et al.* 2012, Matsushima *et al.* 2013). In our study, HAdV D sequences could not be finally assigned to specific HAdV D types, which makes it less likely to find a correlation between HAdV D infection and an individual symptom. In addition, the symptoms induced by HAdV D types are not pathognomonic and can be associated with different pathogens. To determine the effect of specific HAdV D types, it would be necessary, to exclude other pathogens causing similar symptoms and to perform type-specific laboratory analyses.

We analyzed genetic distances to further characterize the HAdV D types involved in this study. Historically, novel AdV types have been determined using serological assays based on recognition of specific epitopes on the viral capsid and on biological properties (oncogenic, haemagglutinating and morphological properties). Nowadays, phylogenetic analyses of complete sequences of the capsid proteins, hexon, fiber and penton base, have been shown to be good predictors for new types and for detection of recombination events (de Jong *et al.* 2008, Maluquer de Motes *et al.* 2011, Singh *et al.* 2012). We analyzed a 4.8 kb long sequence comprising the pV-hexon gene block. Molecular divergence within the hexon protein, the most significant protein for classification and recognition of types, can be used to estimate whether sequences are likely to represent novel AdV types (Ebner *et al.* 2005, Madisch *et al.* 2005). Sequencing of the hyper-variable Loop 2 region of the hexon gene has been proposed to be sufficient for HAdV-typing (Madisch *et al.* 2005). For HAdV D, a genetic divergence ranging from 0.3 to 2.7 % has been reported (Ebner *et al.* 2005). Although analysis of the pV-hexon sequence alone does not permit definite typing of HAdV D, since recombination may have occurred in other genes, comparison of minimum genetic distances between recognized types and the study sequences strongly suggested that novel HAdV D types are involved in our study (*Figure 7; Table 13)*.

5.2 AdV in mammals from rural Côte d`Ivoire (Pauly *et al.* 2015)

This is the first study investigating the prevalence and diversity of AdV shed by diverse domestic animals in Côte d`Ivoire. The aim of the study was to primarily assess the general occurrence of mastadenoviruses by generic PCR, secondarily to further characterize the detected types by phylogenetic and recombination analyses and finally to identify factors which influence AdV shedding by statistical modelling.

Prevalence of AdV: comparison with other countries and implication. AdV shedding is highly prevalent in domestic animals from the Taï region. Reasons for the lower AdV detection rate with the DPOL PCR (3.9 %) compared to the Animal-HEX PCR (21.6 %) could be the longer length of the target fragment of the former (approx. 600 bp compared to 160 bp). Moreover the DPOL PCR might be less generic than the Animal-HEX PCR, as this PCR system was primarily designed to detect primate AdV. The hexon, as well as the polymerase gene is well conserved among mastadenoviruses. Most of the primers used in other studies target the hexon gene, which comprises species- but also the type-specific antigens and is thus perfectly suited for studies, which do not only aim at detecting the virus family, but also at characterizing the detected strain. Therefore a high diversity of animal hexon gene sequences is available in Genbank, and we restricted our further phylogenetic, recombination and statistical analyses on the results of the Animal-HEX PCR.

At first glance, the prevalences found in the current study appear to be much lower than those described for the same animal species in studies from other countries. However, incongruent study designs referring to sample material, detection method and study population limited the feasibility of direct comparison of our cross-sectional study to other studies (*Table 3)*. Moreover, one has to consider that data on AdV prevalence in humans and animals are scarce, particularly concerning AdV in Africa. Most of the data available depict AdV prevalence in humans, non-human primates and chickens and only few of the studies are based on AdV detection by PCR. Direct comparison of AdV shedding prevalence was only feasible for monkeys, pigs and cows, as for these animal species PCR-based studies were available (*Table 3)*. The very limited amount of monkeys, pigs and cows tested (n=7, n=24 and n=14, respectively) might partly explain the prevalence difference and a prevalence obtained from a larger dataset would possibly more correctly mirror the actual situation in the study region. The little information available on AdV prevalence in other domestic animals is merely based on serological studies (*Table 3)*. The interpretation of such serological tests is impeded by the fact that, contrary to PCR, a positive result implies either an active AdV infection or a past AdV infection, unless immunoglobulin G and M are differentiated. Moreover cross-reactions in neutralization assays between different ruminant AdV often prevent accurate type identification (Lehmkuhl *et al.* 2008). When serological results are compared to PCR results, one must take into consideration that cross-reactions between other closely related AdV species cannot be excluded. For dogs, sheep and goats, only seroprevalences were available from other countries and thus direct comparison to the found prevalence detected in the present study (dog: 28 %, sheep: 18 % and goat: 17 %) was impractical for the reasons outlined above. Furthermore, the applied serological methods are often more specific (e.g. ELISA detecting only canine AdV) than the generic PCR system applied in the current study and did likely not detect unexpected AdV species. This referred particularly to dogs and other carnivores, which are normally only tested for canine AdV. However in the present study, no recognized canine AdV were identified, although 28 % of the dogs were found positive. Canine AdV, which cause laryngo-tracheitis and hepatitis in dogs and other wild canine species, are widespread and seroprevalences have been determined for several domestic and wild canine species in various

regions of the world world (Mochizuki *et al.* 2001, Levy *et al.* 2008, Philippa *et al.* 2008, Akerstedt *et al.* 2010, Qin *et al.* 2010, Balboni *et al.* 2013) (*Table 3)*. An explanation for the absence of canine AdV could be the detection method and the tested sample material. In contrast to many studies, which performed canine AdV-specific ELISA of serum samples, we performed a generic nested PCR of rectum swab samples. Rectum swabs, faeces and urine are accurate materials for AdV detection during acute infectious, as well as post-infectious phase (Chaturvedi *et al.* 2008). However, it is possible that antibodies can be detected even longer. It is unlikely that inhibitory substances, which are often present in fecal samples, have prohibited the detection of canine AdV, as other AdV types could be successfully detected. Furthermore, one has to take into account the relatively small number of samples tested in this study (dog: n=58; sheep: n=50; goat: n=60; cow: n=14; pig: n=24; monkey: n=7): failure to identify a specific AdV cannot be interpreted as evidence against its presence, but might be simply due to a very low prevalence.

The study result might be of interest for future rabies vaccination programs. In fact, a canine AdV-2 vaccine that expresses the rabies virus glycoprotein (CAV2-RG) and that can be administered orally, has been developed (Hu *et al.* 2006, Zhang *et al.* 2008) and it has been shown that pre-existing antibodies against CAV reduce the resulting immunity against rabies (Wright *et al.* 2013). As no canine AdV were shed by dogs in the study region, one can assume that this vaccine might be an interesting way to reach also the mainly free-roaming dogs during vaccination campaigns. Rabies is still endemic in Côte d`Ivoire and in many neighbouring countries (Dodet and Africa Rabies Bureau (AfroREB) 2009, Ouattara *et al.* 2012) and oral vaccination would facilitate effective vaccination coverage in rabies control programs. Nevertheless, before a broad application of the vaccine in the study region is instigated, a screening for neutralizing antibodies against canine AdV in dogs of the Taï region is required to exclude circulation of canine AdV.

Ruminant AdV belong to two genera: *Mastadenovirus* and *Atadenovirus* (Benkö *et al.* 1998, Harrach *et al.* 2011). As the primers applied (Animal-HEX PCR) are generic for mastadenoviruses and do not detect atadenoviruses, we assumed that this might have led to an underestimation of the actual prevalence of all ruminant AdV. Further investigation of ruminant and avian atadenovirus shedding by the application of the generic primers of Wellehan et al, 2004 (generic DPOL-PanAdV PCR) did not illuminate this problem. These degenerate primers are supposed to detect AdV from nearly every genus (Wellehan *et al.* 2004). Nevertheless, with this PCR system, we could confirm only a small proportion of the samples which were found positive for ruminant mastadenoviruses with the Animal-HEX PCR primers (11/20, 55 %) and no atadenovirus could be detected, neither in ruminants, nor in chickens. Application of specific atadenovirus primers would clarify if effectively no atadenoviruses circulate in the study region or if low prevalence of atadenovirus shedding remained hitherto undetected.

Co-infection, recombination and potential transmission routes. Precise type assignment of the few bovine mastadenoviruses obtained (n=8/11) with the DPOL-PanAdV PCR was not feasible, as complete genome and polymerase gene sequences of ruminant AdV are lacking in Genbank. If the inconsistency between the obtained polymerase and hexon gene sequences was due to mixed infections with different AdV types, to recombination or if it was simply due to the lack of comparable data, could not be elucidated in the present study. Moreover, it is possible that the target region within the polymerase gene is too well conserved among ruminant AdV to permit definite type identification. Mixed infections have been repeatedly described for other animal species (Balboni *et al.* 2013, Kajan *et al.* 2013). Furthermore, as bovine AdV-2 has been frequently detected in sheep and as antibodies against this AdV (or related AdV) are wide-spread in sheep flocks

(Belák *et al.* 1974, Lehmkuhl *et al.* 2008), mixed infections with caprine or ovine AdV and bovine AdV may occur. Also in the present study, there was evidence that mixed infections with different AdV species occur. In one goat a porcine AdV was detected with the DPOL-PanAdV PCR and an ovine AdV with the Animal-HEX PCR and in another goat a HAdV D was detected with the DPOL-PanAdV PCR and an ovine AdV with the Animal-HEX PCR. However, a critical interpretation of these potential co-infections is required, as only unassertive type assignment was feasible for these sequences due to uniqueness of the sequence (low pairwise identity to the next closely related AdV sequence available in Genbank) or bad quality of the chromatogram. Definite evidence for co-infection was obtained from a piglet (PGAH389), in which a HAdV D and a porcine AdV-3 were detected with the long distance PCR system (LD-HEX-MastAdV PCR) *(Figure 10)*. As co-infection with different AdV types is a prerequisite for natural recombination and as HAdV-D recombinants have been frequently detected in humans (Robinson *et al.* 2011), one can assume that recombination might occur between different animal AdV or even between animal and human AdV. The close genetic relationship between ruminant AdV {e.g. ovine AdV and bovine AdV-2 (Barbezange *et al.* 2000)} might further facilitate homologous recombination. Contrary to HAdV, recombination has been rarely described for animal AdV. There is evidence that the porcine AdV-5 (Nagy *et al.* 2002) resulted from a recombination event between a mastadenovirus and an atadenovirus, and the duck AdV-1 between an aviadenovirus and an atadenovirus (Harrach *et al.* 2011). Of great significance might be our finding that animals can be coinfected with human and animal AdV strains, as recombination involving human and animal AdV strains might have dramatic consequences for public health. This unexpected and enthralling result will be discussed in depth in the last section of the discussion (*5.4 Risk factors for zoonotic transmission of pathogens and zoonotic and interspecies transmission of AdV in Côte d`Ivoire*).

Besides the co-infection and recombination problem, the high diversity and prevalence of AdV shed by ruminants certainly favors virus spread. This might be of importance when the animals are moved during transhumance in search of pastures and watering points or for the largely unregulated international trade. In Togo, another West African country, more than 75 % of the cattle are imported mainly from neighboring countries (Dean *et al.* 2013). Equally, discussion with livestock owners in the study region revealed that many small ruminants and chickens are imported from Burkina Faso. Hence, there is a high risk for disease introduction and spread via the common trade routes. AdV introduction might be facilitated by the fact that AdV are very stable and can resist to temperature and pH changes and to lipid solvents and disinfectants (Harrach *et al.* 2008). As a result, porcine and bovine AdV have been frequently found in water samples (Hundesa *et al.* 2006, Viancelli *et al.* 2012). One can assume that the observed high frequency of AdV excretion in the study region leads to frequent contamination of drinking and river water with animal AdV. Contaminated water might contribute to rapid virus spread. To ascertain the role of water for virus spread, water samples from different origins could be screened for AdV and detected AdV strains characterized in order to illuminate the source of pollution (Wolf *et al.* 2010).

In the current study, no statements could be made as to the role of AdV transmission from wildlife to domestic animals, but the proximity of the Taï National Park certainly increases the risk for such cross-species transmission events. Pathogen transmission from domestic animals to wildlife and vice-versa is facilitated if people live within the borders of national parks and thus if domestic animals and wildlife are sympatric. Examples for diseases which originated in the wildlife population and play a role for both wildlife and livestock, are Foot and Mouth Disease, African swine fever, Trypanosomosis, Heartwater, Bluetongue, etc.. In the present study, cross-species transmission of AdV was frequently observed between small ruminants: sheep shed caprine and goat ovine AdV.

Ruminant AdV were also detected in rectum swabs from dogs. Cross-species transmissions of AdV between different ruminant species have been reported before. Sheep, deer and camelids can be naturally infected with bovine AdV, which may cause enteric and respiratory diseases (Belák *et al.* 1974, Barbezange *et al.* 2000, Lehmkuhl *et al.* 2008, Intisar *et al.* 2010). In fact, although AdV are mainly host-specific and have most probably co-evolved with their animal hosts (Benkö *et al.* 2003, Davison *et al.* 2003), cross-species transmission occurs. Ruminant AdV represent an exception to the co-evolution hypothesis of AdV and were assigned to several genera (*Mastadenovirus* and *Atadenovirus*). A hypothesis from Harrach et al. was that ruminant AdV adapted later to the present-day host (Harrach 2000). The present study provides concordant evidence in favour of common cross-species transmission between the closely related ruminant AdV and the more distantly related dogs. On the other hand, the isolated situation with limited connection to the rest of the country and the absence of wild canines, which could serve as AdV-carrier and -transmitter might also explain, why canine AdV were not detected. The problem of AdV transmission between sympatric wild and domestic carnivores has been approached in Italy, Ethiopia and in Scandinavia, where foxes or wolves are infected with canine AdV strains similar to dog strains (Akerstedt *et al.* 2010, Balboni *et al.* 2013). The same was not observed in the Bale Mountains National Park in Ethiopia, where the seroprevalences of canine AdV in wolves, sympatric domestic dogs and urban dogs were investigated. Although seropositive Ethiopian wolves, as well as urban dogs were identified, all the sympatric dogs, living in the National Park were seronegative. Thus the source of canine AdV in Ethiopian wolves remained unclear (Laurenson *et al.* 1998). Pathogens that can affect both wild and domestic animal species might become a problem for the conservation of endangered species. The findings presented here clearly demonstrate that more comparable studies are needed to complete the knowledge on the molecular diversity of AdV in animals worldwide, on the occurrence of mixed infections and of recombination events.

Detection of novel AdV types and phylogenetic relation. A shortcoming of the very sensitive Animal-HEX PCR primers was that the obtained PCR products were too short and the target gene region too well conserved to permit definite type identification of the detected AdV. With the long distance PCR (LD-HEX-MastAdV PCR) further characterization of the AdV was feasible. Reasons for the lower rate of AdV detection (32 %; 15 out of 46), was probably due to copy numbers being too low in rectum swabs for amplification of approx. 2.7 kb, or to poor sample quality. Phylogenetic analyses, as well as molecular comparison revealed that the detected animal AdV might represent novel types of recognized AdV species (*Figure 10)*. One criterion for species designation requests 5-15 % of amino acid sequence difference (Harrach *et al.* 2011). Hence there is strong evidence that the sole detected SAdV (MTAI277) might not only be a new type, but the first strain of a novel species (pairwise observed genetic distances to every known simian and human AdV was >10 %). Closely related was the SAdV-18, which was isolated previously from fecal samples of asymptomatic rhesus macaques (*Macaca mulatta*). SAdV-18 shares not only the phylogenetic clade *(Figure 11)*, but also molecular characteristics (e.g. 2 fiber genes) with the HAdV F types, which cause gastroenteritis in infected humans.These findings were interpreted as indication for probable cross-species transmission of SAdV-18 between humans and monkeys (Roy *et al.* 2012). The SAdV sequence in the current study was detected in a mona monkey (*Cercopithecus mona)*, belonging to the habituated mona group living in Taï village. Hence close contact to humans is certainly given and one can assume that this SAdV might be transmitted from the animal host to the local population. Close contact between caring humans and captive NHP in research centers or sanctuaries has already resulted in cross-species transmission of AdV: an AdV closely related to HADV F was detected in a sick gorilla in a sanctuary (Wevers *et al.* 2011) and a researcher at the California National Primate Research Center (CNPRC) was infected with the same AdV (Titi monkey AdV) causing fatal pneumonia in a closed

colony of New World monkeys (titi monkeys; *Callicebus cupreus*) (Chen *et al.* 2011). Phylogenetic analysis of the nearly complete hexon gene confirmed the phylogenetic relation of the SAdV (MTAI277) to both, HAdV F and SAdV18 *(Figure 10)* and recombination analyses, revealed potential recombination events involving study sequences. However, these events were not well supported by all the recombination detection methods embedded in the program. To investigate the prevalence and pathogenicity of the detected SAdV for humans and animals, specific screening of the remaining monkeys of the group and of the population of Taï village would be desirable.

Factors affecting AdV shedding. Besides the predictors included in our study (age, gender and animal species), there are likely to be many other factors that affect AdV shedding and which have not been included in this study (conditional R^2=0.18). Such factors could be immunosuppression, coinfection with other pathogens or pregnancy, as it has been shown that these factors often exacerbate AdV pathogenicity and might affect AdV shedding. However we found that, contrary to humans (Pauly *et al.* 2014), gender had a significant effect on infection status of animals. The relatively higher AdV shedding observed in female as compared to male animals ($z=-2.6$, $p= 0.01$) might be due to the immunosuppressive effect of repetitive gestation and lactation *(Figure 13)*. Similarly, in chickens increased AdV shedding during egg production has been reported and might favor vertical transmission of FAdV (McFerran *et al.* 2000). Immunosuppression in humans and chickens has been reported as one of the factors, which increase the pathogenicity of AdV infection and which lead to prolonged AdV shedding (Kojaoghlanian *et al.* 2003). Unfortunately, we could not include “chickens” into the model, as far more hens than cocks were sampled. Also discordant to the human study, age alone did not play a significant role. In humans, younger individuals shed HADV D significantly more frequently and it was derived that exposure to HAdV D likely occurs early in life (Pauly *et al.* 2014). In the present study, the age of the animals was estimated from the physical appearance and only 2 age groups were defined: adult and juvenile. It cannot be ruled out, if a more precise classification in more age groups, similar to the human age groups, would have led to a different result. The variation in AdV shedding between the villages might be partly due to the proximity of the Taï National Park. The villages with the highest AdV prevalence (Gouleako and Pauleoula) in animals are also those, which are closest to the national park entry (*Supplementary Figure 1)*. Direct contact to infected wildlife, but also indirect contact via contaminated water sources might play a role for AdV spread. These villages are also those with the worst connection to the outer world and which have no electricity and current water supply. In contrast to animals, a significant difference in AdV shedding between the villages was not observed for the humans.

The short clinical examination, preceding every sampling, revealed that most of the animals appeared to be in good health (good coat and nutritional conditions and had a normal body temperature). Although the samples were collected by a veterinarian (M. Pauly), who presumably was able to realistically assess the health status of the animals, mild symptoms could have been overlooked. Even discussions on animal health with the owners were mostly non-informative, as the local husbandry conditions make continuous observation of the animals highly impractical. Furthermore, owners probably ignored and underestimated many clinical symptoms and if not, they may rather slaughter sick animals, than wait for convalescence and present them for sampling. Another point is that unhealthy animals might not survive very long in this harsh environment, especially without any veterinary medical care provision (*results section 4.1 Risk factors for zoonotic disease transmission in rural region of Côte d`Ivoire*). Taken together, these considerations suggest that the animal health status was likely over-estimated. Longitudinal studies with long observation phases would provide more information about the current animal health status in the region. This would moreover be of great interest with regard to public health as the negative impact of bad

animal health on public health and wealth is certainly not negligible. Moreover there is certainly need to instigate veterinary services in the study region to increase the awareness of zoonotic diseases and the capacity for disease detection and prevention in animals.
Similarly to the humans, no significant association could be found between AdV shedding and health status. This result was expected, as AdV shedding has been frequently observed in asymptomatic animals (Roy *et al.* 2009) and humans (Horwitz *et al.* 2007). However as porcine, caprine and ovine AdV have also been isolated from animals with pneumonia, pneumo-enteritis or encephalitis (Edington *et al.* 1972, Ducatelle *et al.* 1982, Elazhary *et al.* 1985, Pommer *et al.* 1991, Lehmkuhl *et al.* 1999, Olson *et al.* 2004), a pathogenic potential of these AdV cannot be foreclosed.

5.3 AdV in poultry from rural Côte d`Ivoire (Pauly *et al.* 2015)

Many studies have investigated the prevalence and pathogenicity of Avian AdV in birds from Asian, European or American countries and the prevailing notion of *Aviadenovirus* evolution was based on phylogenetic analysis of those sequences. This is the first study that investigated the occurrence, diversity and phylogeny of Avian AdV shed by poultry in Sub-Saharan Africa.

Prevalence and diversity of the detected FAdV. A high prevalence of FAdV shedding was detected in chickens in the Taï region. The fact that the FAdV prevalence was slightly higher when using the DPOL-PanAdV PCR (58.2 %) than the short HEX-FAdV PCR (42.9 %) illuminates the high sensitivity of this PCR system for the genus *Aviadenovirus*. Although the same infection status (FAdV positive or negative) was obtained for nearly every chicken with both PCR systems, the identified FAdV species occasionally differed. Mixed infections with different FAdV serotypes or even FAdV belonging to different FAdV species have been observed before (Kajan *et al.* 2013) and may be a common feature of FAdV infections. Contrary to other African countries (e.g. South Africa and Nigeria), where chickens were seropositive for the atadenovirus, Duck AdV-1, no atadenovirus was detected in the Taï region. However this is the first report of siadenovirus infection in an African country *(Figure 12).* Although FAdV infections occur worldwide, precise and recent data on diversity and prevalence are only available for few countries. Similar as for the humans and domestic mammals, the incongruent study design in respect to sample material, detection method and study population, excluded a direct comparison of study results from different countries. On the phylogenetic tree, the clustering of the study strains was clearly not geographically based: they formed clades with FAdV from different regions of the world. So far, contradictory statements about the potential correlation between geographical origin and phylogenetic relationship have been published (McFerran 1997, Marek *et al.* 2010). FAdV species D and E were among the predominant species in every investigated country and species A, B and C were only rarely reported (Ojkic *et al.* 2008, Ojkic *et al.* 2008, Pizzuto *et al.* 2010, Lim *et al.* 2011, De Herdt *et al.* 2013, Kajan *et al.* 2013). Considering that FAdV D and E were also the most frequently detected species in the present study, one can hypothesize that members of these species might play a role as commensal or as pathogen for chickens worldwide.

Shedding of avian AdV: implications for animal health and virus spread. Interpretation of FAdV shedding with regard to pathogenicity of FAdV is impeded by the fact that FAdV shedding has been reported in symptomatic and asymptomatic chickens (Meulemans *et al.* 2001). Hence, analysis of cloacal swabs alone is insufficient to definitely identify the causative agent of a chicken disease. However, it might serve as initial analysis. If pathogenic FAdV infection is suspected, further investigation of tissue samples from deceased poultry should follow (Hess 2000). Additional testing for other poultry disease (e.g chicken anemia virus and infectious bursal disease virus) might be of importance, as most of the AdV-induced symptoms are non-pathognomonic and as multiple agents might be involved in the pathogenesis process (Toro *et al.* 1999, Toro *et al.* 2001). Moreover, the analysis should be completed by pathogenicity determination of the respective FAdV isolate since virulence can vary within the same serotype (Okuda *et al.* 2006, Beach *et al.* 2009, Lim *et al.* 2011).

Even though several disadvantages of cloacal swabs as test material have been highlighted, this approach is well suited for quick determination of FAdV occurrence and diversity and thus for attainment of the study objectives. As a matter of fact, FAdV can be isolated from the gatsrointestinal tract longer than from other tissues (shedding duration was estimated to 18-21 days (Cook 1983) and is age-dependent (Clemmer 1972). Moreover, similar to AdV in humans (Garnett *et*

al. 2009), there has been evidence that AdV infections of poultry become latent and that reactivation with viral shedding occurs in situation of stress and immunosuppression (Girshick *et al.* 1980, Beach *et al.* 2009). Hence, the present analysis was limited to assessment of FAdV prevalence and diversity and thorough correlation between pathology (observed mild respiratory, dermatologic or orthopedic symptoms) and FAdV shedding could not be determined. However one can suspect that at least a part of the detected strains might have caused disease in the study chickens, as some study sequences were closely related to pathogenic and others to non-pathogenic FAdV strains. The applied PCR system targets the hexon gene. For species and type identification the hexon gene is the standard PCR target, but for the distinction between virulent and avirulent FAdV the long fiber gene might be a better choice (Okuda *et al.* 2006, Palya *et al.* 2007, Marek *et al.* 2010). The pathogenic role of the siadenovirus, TAdV-3, detected in chickens from several villages was also not assessed. TAdV-3 can cause hemorrhagic enteritis (HE) and immunosuppression in turkeys, and is the causative agent of the Marble Spleen disease (MSD) in pheasants and of the Avian AdV Splenomegaly (AAS) in chickens. All three diseases are caused by three serologically and genetically indistinguishable isolates of TAdV-3 (Pitcovski *et al.* 1998) (Palya *et al.* 2007). Similar to most FAdV infections, virulent, but also avirulent TAdV-3 infections have been observed in chickens and turkeys and several factors, as for instance stress, can affect the pathogenicity in the animals (Gross and Domermuth 1988, Gross *et al.* 1988). However as the strain detected was highly similar to a virulent field strain, a potential pathogenicity of the study strain can not be excluded (99 % similarity to the already published virulent Hemorrhagic enterititis Virus (HEV) (Pitcovski *et al.* 1998); Genbank accession N° AF074946.1). Histological and pathological analysis of spleen samples from chickens of the region would clarify the pathogenic role of TAdV-3. The absence of atadenovirus is promising, as the atadenovirus Duck AdV-1 is transmitted from waterfowl to chickens, where it causes the Egg-Drop syndrome (EDS), characterized by dramatic drop in egg production (Van Eck *et al.* 1976). As eggs are frequently processed in the traditional dishes *(Results section 4.1 Risk factors for zoonotic disease transmission in rural region of Côte d`Ivoire)* and are important protein and cash sources, occurrence of Egg-Drop syndrome would have severe consequences for the local poultry farming. A longitudinal study including explicit collection of clinical and epidemiological data, as well as the collection of tissue samples would reveal more insights into the clinical importance of avian AdV infections and potentially confirm the role of avian ADV in various disease conditions.

Although the pathogenic role of FAdV was not investigated, our study showed that a remarkable high diversity of FAdV serotypes circulated in this rather isolated region of Côte d`Ivoire (*Figure 14* and *Figure 15*). This result disproves the hypothesis that the low diversity detected in Japan and Australia, can be explained by their isolated geographic situation (Kajan *et al.* 2013). However discussions with local breeders revealed that part of the local chickens are imported from other African (e.g. Burkina Faso) and European (e.g. France) countries. Thus, the geographic situation of the study region might be less isolated than assumed at first glance. As several studies suggested that commercial hatching might contribute to the spread of avian AdV from one country to another (Adair and Smyth 2008), importation of AdV-infected chickens might represent one factor contributing to the high FAdV prevalence and diversity detected. Natural migration of birds between Europe and Africa might also enhance the spread of AdV, as it is known that migrating wild birds play a role in the spread of various avian viruses and as particularly wild waterfowl can be reservoir of diverse avian pathogens, including avian AdV (Ziedler *et al.* 1995, Waldenstrom *et al.* 2002, Hlinak *et al.* 2006).The proximate Taï National Park is habitat to an elevated avian biodiversity and thus to a high diversity of potential hosts of avian AdV. Direct horizontal transmission might occur if wild and domestic birds encounter and indirect horizontal transmission if water is contaminated by feces from wild birds (Hafez 2010). The local husbandry conditions certainly create the perfect environment for direct and indirect

transmission. As chickens are not kept in closed henhouses and roam freely through the villages, intake of AdV-contaminated forage is very probable and contact between chickens from different owners frequent. This facilitates pathogen transmission not only between chickens, but also between different domestic and wild avian species as their habitats overlap (Hopkins *et al.* 1990). The study region represents the perfect environment for interspecies transmission of FAdV, but also of other more severe poultry diseases, e.g. avian Influenza. Another example could be the turkey AdV-3 (TAdV-3) detected in 4 chickens from 3 different villages (Gouliako, Pauleoula and Taï). It remains enigmatic, how this virus reached the rather isolated Taï region as no turkeys are raised in the area and as no TAdV-3 associated disease has ever been reported from an African country. As mentioned above, contaminated material via indirect transmission route, or imported chickens or wild birds via direct transmission route might be the initial infection sources. Although turkeys are the main host of TAdV-3, natural infection in chickens has been previously described and high sero-prevalences were found in Japan and the USA (Domermuth *et al.* 1980, Yamaguchi *et al.* 1982). Implementation of basic biosecurity measures (Ono *et al.* 2007) combined with vaccination of young layer and breeder flocks would not only decrease the risk for development of AdV induced diseases and virus shedding (Heffels *et al.* 1982), but also for the transmission of other major poultry diseases.

New proposal for FAdV classification. As precise characterization of the detected AdV type is useful for epidemiological tracing and vaccine production (Kajan *et al.* 2013), all the positive samples of this first screening were subsequently tested with generic FAdV primers (long HEX-FAdV PCR) established before (Meulemans *et al.* 2001). Not all the criteria for definite FAdV type and species identification, as proposed in the Ninth ICTV report (Harrach *et al.* 2011), could be fulfilled. Hence we decided to adopt other criteria, applied in previous studies. Species and type demarcation based on phylogenetic tree topology and on the criteria proposed by Marek et al. revealed consistent results concerning the identified FAdV species (Marek *et al.* 2010)(*Table 16 and Figure 14)*. Nevertheless, FAdV type assignment was not feasible for some of the study sequences (*Table 16)* and we concluded that these sequences may represent novel FAdV types. To confirm our suspicion and to statistically elucidate FAdV evolution, species delineation was performed. Although the limitations of such evolutionary scenarios based on single locus data are well known, their usefulness for the surveillance of evolutionary patterns has been convincingly shown before (Monaghan *et al.* 2009) and different methods have been developed to permit evolutionary analysis based on a single locus dataset. The Generalized Mixed Yule Coalescent (GMYC) method is a well-recognized method, which has been successfully applied on diverse datasets to delimit independently evolving species. This method is based on the assumption that all species are monophyletic and that all taxa of a species share a common ancestor (Fujisawa *et al.* 2013). In our study, the method only partly confirmed the previously recognized species and many species were further subdivided into several subspecies. Some of the study sequences were even proposed as discrete subspecies *(Figure 15).* In agreement with previous studies (Meulemans *et al.* 2004, Marek *et al.* 2010), the Japanese strain TR22 (FAdV 5) did not cluster with any of the five FAdV clusters and was recognized as discrete species. The original host species of this FAdV isolate is unknown, but it might be a wild bird or non-chicken poultry (Kajan *et al.* 2013). The result of the GMYC analysis challenges the present species concept of *Aviadenovirus*, which is mainly based on serological and biological properties. The question therefore arises whether, as a consequence, one should reconsider the present FAdV taxonomy criteria. Replacing them by more precise criteria based on genomics would perhaps bring more clarity and would certainly facilitate species and type assignment, particularly as now many studies focus on phylogenomics (Ojkic *et al.* 2008, Mase *et al.* 2009, Steer *et al.* 2009, Marek *et al.* 2010, Lim *et al.* 2011, Kajan *et al.* 2013). A revised FAdV taxonomy, based on evolutionary patterns, would comprise

a larger number of species or, alternatively, the current species would be subdivided into several subspecies *(Figure 15)*.

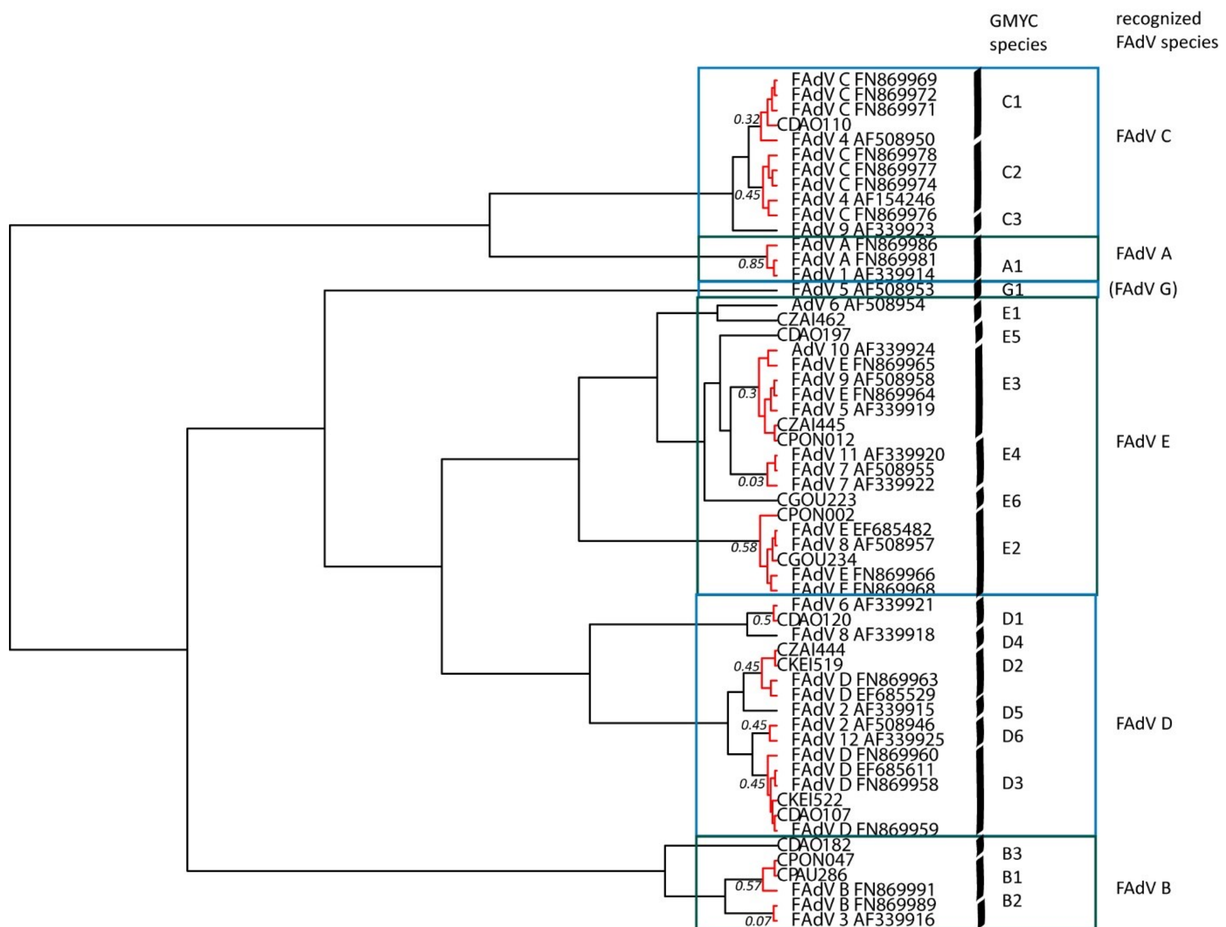


Figure 15. Species delineation of FAdV

Bayesian analysis of a 471 bp long alignment of partial hexon gene sequences, comprising at least one reference strain of every Fowl adenovirus species and sequences identified in this study. A MCMC chain of chainLength 2000000 was run with the substitution model GTR+I+G, under a relaxed lognormal molecular clock and the prior assumption that the population size has remained constant throughout the time spanned by the genealogy. The reference strains are represented by host name, type and Genbank accession number. The study sequences were all detected from chickens cloacal swabs (code: first letter “C”). Adenovirus is abbreviated to AdV. The species delineation was run applying the Generalized Mixed Yule Coalescent (GMYC) method. The single-step method revealed to be significant (p-value=0.04) and the number of species clusters was estimated to be 12 (confidence interval: 3-16). The proposed species clades were distinguished by red and black branches. The brackets represent the proposed subspecies within the recognized species and based on the delineation analysis.The coloured boxes represent the different recognized adenovirus species. AIC weights are only shown if the obtained values supported the proposed delination.

4.6Risk factors for zoonotic transmission of pathogens and zoonotic and interspecies transmission of AdV in Côte d`Ivoire [(Pauly *et al.* 2015) and (Mossoun *et al.* 2015)

4.7

Risk factors for disease transmission: sociological and behavioral aspects. Observations of the living conditions of people and animals, together with the statistical analyses of the questionnaires revealed many risk factors for zoonotic disease emergence *(Table 18)*. The overlapping habitat and consequently sustained close contact between various domestic and wild animal species and humans creates many opportunities for cross-species transmission of pathogens. The same was reported for the live bird markets in Southeast Asia, where people, pigs, ducks, geese and chickens intermingle. These were probably the starting point for recombinant human influenza viruses (Ai *et al.* 2013, Fuller *et al.* 2014). Particularly, pathogens of NHP are of major concern for human health as the close phylogenetic relationship between humans and NHP facilitates pathogen exchange (Davies *et al.* 2008). Hence, the close and prevailing contact to habituated monkeys and the frequent activities implicating contact to animal body fluids and tissues (butchering, handling, preparing, smoking etc.) *(Table 10 and Table 11)* increase the risk for zoonotic disease transmission. Besides the frequency of exposure, the high prevalence of potentially zoonotic pathogens in NHP from Taï National Park is another factor increasing the likelihood of cross-species transmission and of disease emergence (Leendertz *et al.* 2004, Leendertz *et al.* 2008, Morozov *et al.* 2009, Leendertz *et al.* 2010, De Nys *et al.* 2013). The fact that a group of habituated mona monkeys (*Cercopithecus mona*) lives in Taï, might increase the risk for introduction of pathogens from wildlife and for pathogen transmission to women and children. Habituated monkeys in Asian temples often harbor viruses with zoonotic potential (e.g. Herpesvirus B and SFV) and transmission of these viruses during close contact to human visitors, as well as through occasional bites and scratches has already occurred (Engel *et al.* 2006). But even in the other villages of the Taï region, children are at risk as there seems to be no major difference in NHP consumption between adults and children (approx. 60 % of the children eat monkeys and 15 % chimpanzees).

The analysis of the demographic data from the study participants (*Table 4 and Table 5)* confirmed that over the last decades many immigrants from neighboring countries and of various ethnic groups have settled in this fertile region to work in the rubber plantations (48 % of the study participants originated from Burkina Faso and grower was the main occupation of male study participants). This wave of migration has not only created dramatic land conflicts (International Crisis Group 2014), but might have generated the perfect environment for the emergence of new pathogens. Migration has been repeatedly associated with disease emergence (Gushulak *et al.* 2009) as on one hand, immigrants can be carriers of “new” pathogens and on the other hand, they have not developed immunity to endemic pathogens (Karesh *et al.* 2012). It would be of interest to investigate in the Taï region the occurrence of diseases, which commonly emerge after intense migration {e.g. tuberculosis (Zammarchi *et al.* 2014)}. An interesting finding was that participants born in CI consumed significantly more monkeys and chimpanzees than particpants born in BF ($p<<0.01$) (*Table 11 and Figure 6)*.The differing dietary habits between the birthcountries and between the ethnicities can probably be explained by the differing traditional lifestyles (pastoralism versus bushmeat hunt), which were shaped by the habitat (low versus high biodiversity). Furthermore, diverging religious faith (Muslims versus Christians) might influence the eating behavior (abstinence from or consumption of pork/porcupine meat). Similarly, significantly more bushmeat was consumed in forest sites than in savanna in Cameroon (Wolfe *et al.* 2004). The finding also rejects the hypothesis of Refisch, et al. that migrants hunt animals, which represent taboos (in local French “*totems*”) for the local people (Refisch 2005). Moreover, the original territory of the ethnic group (*Ethnicity: origin)*

was found to be significant ($p<<0.1$): second-generation immigrants (children of those, who were born in Burkina Faso and have relocated to Côte d`Ivoire) did not only claim to belong to an ethnical group from BF, but also kept the nutrional habits of their ancestors, even though they have possibly never been in their country of origin.Hence, there is no or only little adaptation to local nutritional habits after migration.

The difference between the villages was less obvious *(Table 11)*. Nevertheless, different facts affirm the observed differences in contact rate to NHP bushmeat. Taï and Keibly are the largest villages of the region. Furthermore, it is known that Taï constitutes a hub for NHP bushmeat imported from Liberia (Covey 2014) and that the amount of poaching signs increased particularly in the Northern part of the Taï National Park, adjacent to Keibly between 2005 and 2012 (Yapi *et al.* 2011, N'Goran *et al.* 2013) *(Table 11)*. The bushmeat market of Keibly might also provide the villagers from Zaipobly and Gahably, which are both situated relatively close to Keibly *(Supplementary Figure 1)*. However, as mentioned before, direct comparison of the data obtained in Taï with those of the other villages is not entirely correct. As a matter of fact the data are biased as in Taï only women working in the local restaurants (*"maquis"*) were questioned, whereas in the other villages everyone could volunteer to participate in the study.

To recapitulate, we observed in this ecotone {"*transition zone between adjacent ecological systems*" (Jones *et al.* 2013)}, many of the previously identified major drivers for the emergence of zoonoses *(*Table 18*)* (Karesh *et al.* 2012, Jones *et al.* 2013).

Comparison to other studies: possible explanation and consequences. In 2006, samples and comparable behavioral data on bushmeat contact (mainly NHP contact) were collected from humans in the Taï region (Calvignac-Spencer *et al.* 2012). While interpreting and comparing the questionnaires, it is important to consider that officially hunting of wild animals is forbidden in CI since 1974. Consequently, the data might contain some bias, as some participants might have been reluctant to provide accurate information on their bushmeat-related activities. However, as this bias is probably similar for each study, we assumed that comparison of the study findings should at least accurately reflect the current tendencies *(Table 17)*. Furthermore, in 2004 data on exposure to NHP were collected from remote Cameroonian villages in different ecosystems (Savanna, Lowland forest, Gallery forest) (Wolfe *et al.* 2004).

We observed a clear pattern concerning the activity allocation: while men are responsible for hunting and dismembering, women predominantly prepare and cook bushmeat *(Table 11 and Figure 6)*. Men consume and hunt significantly more monkeys and chimpanzees then women ($p<<0.01$; 0.3 % of the women and 19.5 % of the men reported to hunt NHP; 54.8 % of the women and 39.1 % of the men reported to cook NHP) (*Table 11 and Figure 6)*.The same pattern has also been observed in Cameroon and in CI in 2006 (Wolfe *et al.* 2004, Calvignac-Spencer *et al.* 2012) *(Table 17)*. A finding of Calvignac et al. further supported that men constitute the major risk population for infections with primate pathogens: the majority of the persons, who were infected with viruses closely related to STLV-1, were men (Calvignac-Spencer *et al.* 2012). Similarly, in a study investigating the cross-species transmission of SFV from NHP to humans in Gabon, anti-SFV antibodies were only found in men (Mouinga-Ondeme *et al.* 2012).This finding also underlines the disparity between men and women in this rural region and that discrimination of women with regard to nutrition seems to be still deeply rooted in local tradition (Food and Agriculture Organization of the United Nations 2014).

Moreover in the three studies, a similar cascade was observed concerning the percentages of people reporting doing a certain NHP-related activity: from a relatively high percentage reporting to eat (86 %), to a low percentage reporting to hunt (9.9 %) NHP *(Table 11, Table 17 and Figure 6)*. As the transmission of blood-borne pathogens is more likely during the hunt and the slaughter of NHP than during consumption of cooked meat (Wolfe *et al.* 2005), only a small proportion of the population might be at high-risk. This is further supported by our finding that considerably more persons reported contact to monkeys than contact to chimpanzees, as the risk for zoonotic transmission is highest during close contact to great apes (e.g. chimpanzee, gorilla) (Calattini *et al.* 2007, Mouinga-Ondeme *et al.* 2012). In the present study, noticeably 1.2 % of the study participants reported having been injured by a NHP in the past. If the risk for zoonotic transmission of SFV via monkey and chimpanzee bites is comparable to Cameroon (35 %) (Calattini *et al.* 2007), one can hypothesize that 0.4 % of the study participants might be SFV-seropositive. However, the amount of infected people might be even higher as other NHP-related activities, comprising contact to live NHP or to NHP body fluids (e.g. cutting or manipulating the meat and carcasses, consuming uncooked meat, keeping NHP pet) might also represent a risk for retrovirus transmission (Hahn *et al.* 2000, Switzer *et al.* 2004, Calattini *et al.* 2007).

Comparison of the behavioral data of 2006 (Calvignac-Spencer *et al.* 2012) and of the present study revealed a general decrease in exposure to NHP bushmeat *(Table 17)*. In 2012 fewer male participants reported that they dismember (63 % in 2006 versus 25.4 % in 2012), prepare/cook (44.3 % in 2006 versus 39.1 % in 2012) and eat (86.1 % in 2006 versus 63.9 % in 2012) bushmeat than in 2006. The observed decrease might be a positive consequence of the successful activities of the NGO "Wild Chimpanzee Foundation" (WCF). The activities of the WCF might also have led to a substantial concept change with regard to NHP bushmeat, particularly among the younger generations *(Figure 6 and Table 11)*, which are also the main targets of their actions. Another explanation for the less frequent consumption might be that our field mission took place during the acute political crisis phase, during which access to bushmeat from Liberia was hampered as officially the transboundary bushmeat markets were closed (WCF 2013). Normally, bushmeat is imported daily from Liberia (Covey 2014).

On the other hand however, the political crisis, which lasts since 2002, might also have led to the observed increase in bushmeat hunting (5.8 % in 2006 versus 9.9 % in 2012) *(Table 17)* (Koné 2013, International Crisis Group 2014). A similar scenario has been observed in DRC, where during the armed conflict the local authorities lost control of the bushmeat trade (Draulans 2002, Karesh and Noble 2009, Beyers *et al.* 2011). Additionally, the ONUCI (United Nation Operation in Côte d`Ivoire), based in the region, revised the logging road connecting Guiglo and the villages south of Taï. Taken together, the improved access and the fragile security situation in the region might have led to an increase in commercial bushmeat trade between the rural Taï region and the urban areas.

Moreover, the extent of exposure to NHP determined for persons in Cameroon was also considerably higher. For instance, 25.4 % of the persons reported to dismember NHP in CI in 2012, while the percentage reached 65 % in Cameroon in 2004 *(Table 17)* (Wolfe *et al.* 2005). It would be of interest to obtain current data from Cameroon to elucidate if this decrease in bushmeat consumption is a common trend in rural areas of West Africa.

In studies on SFV, wild pets were not considered to represent a risk, as they are mostly orphans, are separated from any potential infection source and seldomly bite since they are well habituated (Wolfe *et al.* 2004, Betsem *et al.* 2011, Mouinga-Ondeme *et al.* 2012)**.** This might not be true for the

Taï region. Here adult sooty mangabeys (*Cercocebus atys*), but also putty-nosed monkeys (*Cercopithecus nictitans*) and mona monkeys (*Cercopithecus mona)* are kept as pets. Both cercopithecus species can be host to different retroviruses (Peeters *et al.* 2002, Mouinga-Ondeme *et al.* 2012). Furthermore, it is known that sooty mangabeys from the Taï National Park are infected with SIV (Santiago *et al.* 2005) and that both, STLV and SIV, can be transmitted from mangabeys to humans (Hahn *et al.* 2000). As 17.2 % of the study participants reported to keep a monkey as a pet or to be in contact with a primate pet, one can hypothesize that keeping wild pets might represent a considerable risk factor for zoonotic disease transmission. This percentage is also significantly higher than in Cameroon, where only 11 % of the study participants kept NHP pets (Wolfe *et al.* 2004). However, the percentage of our study includes not only people, who reported “keeping a NHP pet”, but also those, who reported just “being in contact with a NHP pet”.

Variable	**Activity resulting in contact with NHP bushmeat**											
	Hunting (%)			**Dismembering (%)**			**Preparing or cooking (%)**			**Eating (%)**		
Year	2006	**2012**	2004	2006	**2012**	2004	2006	2012	2004	2006	**2012**	2004
Country	CI	**CI**	Cameroon	CI	**CI**	Cameroon	CI	CI	Cameroon	CI	**CI**	Cameroon
Women	0	**0.3**	approx. 0	62.4	**11.7**	approx. 70	66.9	54.8	?	81.3	**56.8**	approx. 82
Men	11.6	**19.5**	approx. 48	63.6	**39.1**	approx. 60	21.6	23.4	?	90.8	**71**	approx. 85
All participants	5.8	**9.9**	approx. 24	63	**25.4**	approx. 65	44.25	39.1	?	86.05	**63.9**	approx. 83.5

Table 17. Comparison of the frequency of reported activities resulting in contact with NHP, between 2 countries, CI and Cameroon (Wolfe et al. 2004), and between 2006 (Calvignac-Spencer et al. 2012) and 2012

Consequence of living condition: shedding of HAdV by animals. As a result of the restricted medical and inexistent veterinary provision *(Results section 4.1 Risk factors for zoonotic disease transmission in rural region of Côte d`Ivoire)*, knowledge about hygiene praxis, about risk factors for zoonotic transmission pathways, about disease symptoms in animals and humans and about potential consequences and adapted response in case of outbreaks is limited. This knowledge gap, but also the lack of sounder alternatives becomes obvious as soon one visits a local village: there is no clear separation neither between human and animal areas, nor between feeding and manure or waste areas. Scrapheaps and latrines are frequently located in the center of the villages or adjacent to water wells and incineration is the sole waste disposal method. This might have dramatic consequences, as it has been shown that there is a high risk for viral contamination of wells, if they are situated next to latrines (Verheyen *et al.* 2009). Besides, all of these factors increase the risk for fecal contamination of the environment via runoff, drainage, direct deposition or by contamination through slaughter sewage. Fecally contaminated water represents an elevated public and animal health risk, as it can serve as source of waterborne diseases, e.g. adenoviral diseases. This is particularly true in the study region, given that a substantial portion of the rural population still relies on untreated surface water sources for domestic purposes, and given that humans and animals share water provisions.

For many animal species (e.g. dog and pig), not only ingestion of fecally contaminated water, but also deliberate consumption of feces has been observed. This considerably increases the risk for ingestion of high amounts of enteric viruses. One concrete consequence of the living and husbandry conditions might be the shedding of both, animal and human AdV by small ruminants, pigs and dogs *(Figure 10)*.

Whether animals shedding HAdV can be considered to be reservoir or mixing vessels for HAdV or whether the shedding is only due to the ingestion of contaminated material and following passive passage is not clear. It would be crucial to further illuminate the role of domestic animals as AdV reservoir. Pathogen reservoirs are defined as "one or more epidemiologically connected populations or environments in which the pathogen can be permanently maintained and from which infection is transmitted to the defined target population". It has been shown that understanding the infection dynamic in the reservoir host is necessary to successfully combat the infection in the target population (Haydon *et al.* 2002)

Particularly dogs and pigs, but also other animals roaming through villages, could constitute mixing vessels, because they seem to be susceptible to AdV from different species. The susceptibility to pathogens from diverse hosts favors coinfection with different AdV types and subsequent genetic recombination. A similar scenario has been reported for pigs, which can be infected by both, human and avian subtypes of influenza A, and were identified as source host of some severe influenza epidemics in humans (Ma *et al.* 2008). Mixed infections with different AdV types have been repeatedly observed in the present study, and hints for recombination events were identified.

Besides the reservoir and mixing-vessel scenario, the passive passage scenario can be of importance for human health. Knowing that most of the animals have no restricted territory, animals shedding HAdV might accelerate virus spread within, but also between villages. One could see them not as HAdV host, but as HAdV vector or carrier with an important role as intermediate or amplifier hosts for AdV. By shedding HAdV, animals would contribute to the maintenance of infection in humans and to the spread of HAdV in the region. A similar scenario has been described for domestic cattle shedding human protozoal parasites, such as Cryptosporidium hominis (Duffy and Moriarty 2003). The role for virus spread through diverse wild and domestic carnivores, feeding on infected prey, has also been shown for other foodborne viruses, such as influenza virus (Rimmelzwaan *et al.* 2006). Moreover, it is possible that HAdV evolve in the unintentional animal host and spill back to humans. Pigs have been identified as amplifiers for different human infections and have been implicated in severe disease outbreaks (e.g. Nipah virus) (Chua *et al.* 2000). Carnivores and scavenger have been discussed as sentinel species for diverse pathogens, as it can be assumed that pathogens accumulate in these animal species, since they feed on various prey species and hence might be exposed to all the pathogens circulating in the prey population (Cleaveland *et al.* 2006, Halliday *et al.* 2012). This accumulation effect could also explain the high diversity of AdV species detected in dogs and pigs.

An argument for the passive passage scenario may be that the HAdV species ratio shed by humans corresponds to the ratio found for the dogs: the most prevalent HAdV species being species D. We have shown that a high diversity of HAdV D types circulates in the human population (Pauly *et al.* 2014) and the same seems to apply to the animal population. Comparison of the minimum genetic distances (minGD) of the HAdV D detected in animals with recognized HAdV D types and the HAdV D detected in the human study participants with Genbank sequences revealed that each of the animal sequences was unique *(Figure 9)*. The hexon gene is the most significant protein for classification and recognition of HAdV types. However, similarly to HAdV (Crawford-Miksza *et al.* 1996, Robinson *et al.* 2011), we identified hints for recombination within the loop regions of the hexon gene of AdV

detected in animals. As the hexon is also the target site of the serum neutralization and PCR assays, type identification solely based on serology or blast analysis of partial genomes might be misleading. In the ninth report of the ICTV, AdV serotypes were defined as either exhibiting no cross-reaction with others or showing homologous/heterologous titer ratios of 8 or 16. Moreover, serotypes presenting substantial biophysical, biochemical, or phylogenetic differences are considered to be distinct (Harrach *et al.* 2011). Hence, no threshold value for the phylogenetic difference was set in the ICTV report. It may prove beneficial if precise criteria for typing of animal AdV based on genomics would be proposed. The Human AdV Working Group supports already such an approach for HAdV (Brister *et al.* 2009). Nevertheless, the relatively high minGD values of the HAdV detected in animals in this study suggest that they might represent novel types *(Figure 9)*. Surprisingly, none of these "animal" HAdV D types was identical to the types found in the local population. However, no final conclusion with regard to transmission ways could be drawn from this observation, as only a small proportion of the HAdV D circulating in animals and humans in the study region were compared.

AdV as anthropozoonosis: host range of AdV wider than previously assumed? Contrary to the HAdV D, the HAdV-C2 and HAdV-F41 detected in dogs were highly similar to recognized Genbank sequences. HAdV- 40 and -41 infections can be fatal for immunocompromised individuals (Lion 2014). These AdV are, after noroviruses, astroviruses and rotaviruses, the most common viral pathogen involved in acute gastroenteritis in children (Djeneba *et al.* 2007, Filho *et al.* 2007, Al-Thani *et al.* 2013). Our finding suggests that the generally assumed clear separation between primate (simian and human) and animal AdV might not represent the real phylogenetic relation. Moreover, one might reconsider the standard approach that the original host of an AdV is necessarily the host in which the AdV was first detected: what if the original host of the multi-host "bovine" AdV was e.g. sheep and not cattle and what if the first "human" AdV would have been detected in a dog? Accurate attribution of an original host is complicated for AdV with wider host ranges.There are still many uncertainties concerning AdV taxonomy and phylogeny and a flexible, sceptical and open approach is required for further investigations. Except for the SAdV, only one animal AdV clusters at present with the human AdV species: the bovine AdV-9, detected in a cattle and closely related to HAdV-2 and -5, was assigned to the species HAdV C (Klein *et al.* 1959, Klein *et al.* 1960). It is likely that more "ovine", "porcine", "caprine" and "canine" AdV will be added to the "human" AdV species, if the diversity of AdV shed by animals was investigated in more depth.

Thus far, the majority of the studies investigating reverse zoonotic transmission (from human to animal) focused on bacterial or mycobacterial infections and on cross-species transmission of viral agents between humans and NHP (Köndgen *et al.* 2008, Morgan 2008, Messenger *et al.* 2014). However, cross-species transmission of the neglected viral infections between livestock and humans might have significant health and economic consequences (Messenger *et al.* 2014). The majority of the pathogens infecting domestic animals are considered multihost pathogens (Cleaveland *et al.* 2001) and many originated from wildlife (Daszak *et al.* 2000). Domestic animals can serve as "bridge species" or intermediate hosts facilitating pathogen transfers between wild animals and humans (Daszak *et al.* 2000, Pearce-Duvet 2006, Wolfe *et al.* 2007). In line with our findings, it is conceivable that domestic animals are occasionally challenged with human-derived pathogens, similarly to humans (Wood *et al.* 2012). Likewise, in a comparable study setting in Uganda, gorillas, livestock and humans harbored similar E.coli (Rwego *et al.* 2008). In the Taï region, a high prevalence of extended spectrum beta-lactamases (ESBL)-producing Enterobacteriaceae were isolated from humans and dogs (Albrechtova *et al.* 2014). For HAdV one prerequisite for efficient host switch is fulfilled: the presence of the appropriate receptors on the host`s cells (Parrish *et al.* 2008, Flanagan *et al.* 2012). In

fact, CAR and sialic acid serve as receptor for many human, but also for many animal AdV species (Zhang *et al.* 2005).

The consequences of HAdV shedding on animal health were not ascertained in this study. A swine experimentally infected with HAdV-5 developed symptoms of interstitial pneumonia, comparable to the disease observed in infected immunocomprised humans (Jogler *et al.* 2006) and the tumorigenic effect of experimental HAdV infection in rodents has been shown (Wimmer *et al.* 2010). Hence, pathogenicity of HAdV infections in animals cannot be excluded. However it remains questionable, if HAdV in animals reached the stage of endemic disease, as with the interspecies transmission of a pathogen only the first step leading to endemic disease is fulfilled. To persist in the new host population other factors are critical as defined by Wolfe et al, 2007: "*duration of the host`s infectivity, the rate of infection, rate of development of host protective immunity, as well as host population density, size and structure*" (Wolfe *et al.* 2007). To rule out passive gastrointestinal passage of AdV after ingestion of contaminated material, one could test other animal samples (e.g. gut and lymph nodes to detect also latent infections) to distinguish an acute and a latent infection or current shedding in stool after passive passage. In the present study, besides the rectum swabs, only blood samples from most of the HAdV-positive animals were available. Not surprisingly, HAdV could not be detected from blood samples, neither with generic, nor with specific PCR. Similarly, the infection with titi monkey AdV of marmosets was limited to the respiratory tract and no AdV could be isolated from blood or other tissues (Yu *et al.* 2013). Viraemia was only found in the initial host, the titi monkey and even here only in individuals with fatal pneumonia and hepatitis (Chen *et al.* 2011). Abortive infections of HAdV in animals would explain the discrepancy between HAdV detection in rectum swabs and in blood. Another explanation could be that the respiratory and gastrointestinal tracts are the primary sites of infection and replication of AdV in animals. Equally, HAdV infections in humans are often limited to the gastrointestinal and respiratory tract and antigen detection in blood is feasible only in cases of life-threatening disseminated infection in immunocompromised patients. In such patients the disruption of endothelial intercellular junctions facilitates systemic AdV infection (Zhang *et al.* 2005).

Potential contamination issues were considered while designing the study. Nevertheless, the high sensitivity of the generic semi-nested Animal-HEX PCR system presents advantages and disadvantages. On one hand, it enabled sensitive detection of presumably all present mastadenoviruses; on the other hand, the high sensitivity might have led to false-positive results as nested PCRs are prone to contamination by first round products. However, this disadvantage was ruled out by confirmation of the HAdV positivity of several animals with the long distance PCR (LD-HEX-MastAdV PCR). As a matter of fact, false positive results represent a minor concern for long-distance PCR, because the virus load must be higher for successful PCR. Hence animals which were positive in the LD-HEX-MastAdV PCR probably shed high quantities of AdV. One could also interprete this as first hint for productive infection with e.g. HAdV in animals. The application of quantitative PCR on diverse sample material from animals, shedding human and animal AdV, would be of interest to draw conclusions as to infection progression, severity and to virus distribution. Moreover one could, by quantifying the amount of pathogen shed in feces, assess the risk for fecal contamination of the envionment.

Absence of zoonotic transmission of AdV. A puzzling result of the study was that, contrary to the animals, the human participants did not shed AdV from other hosts. Despite frequent exposure of the local population to blood, organs and feces of the frequently infected domestic animals and NHP (Wevers *et al.* 2011), there was no evidence for zoonotic transmission of AdV.This finding alone

would confirm the hypothesis that AdV predominately co-evolved with their host. Host-specificity is a common feature of DNA viruses, which, in contrast to RNA-viruses, are characterized by large size and low replication and mutation rates. The comparatively slow evolution rate hinders a rapid adaptation to a new host and hence interspecies transmission. Nevertheless, as we did detect HAdV in different animal species, interspecies transmission of AdV certainly occurs, but appears to be "one-way": only from humans to animals and not vice-versa.

Several factors might have contributed to the occasional transmission of HAdV to animals and the absence of reverse transmission. While animals are continuously exposed to material contaminated with human feces, the amount of infectious virus ingested by humans might be reduced by stewing for hours. Another explanation could be the laboratory method applied.The blocking primers used in our study to exclusively and specifically amplify animal AdV and not HAdV D. However, it cannot be excluded that they might have blocked other AdV species, leading to false-negative results. Yet, as the negative results were confirmed with the species-specific PCR systems, such an unintentional blocking is improbable. Furthermore, the improved access to drinking water in the region should be considered as possible explanation. Recently, several covered and well-maintained wells were constructed in several villages. This certainly benefitted the local population by reducing the risk for water contamination with animal waste or rainwater and might have contributed to decrease indirect transmission of AdV. As AdV are very stable and can resist to temperature and pH moderations, they are often used for tracing of fecal environmental contamination and for evaluating the water quality (Sibley *et al.* 2011). Moreover, the pollution source can be determined by identifying the contaminating AdV type (Hewitt *et al.* 2013). It would therefore be of interest to test the water from different wells for AdV to assess, if in the study region well water still represents a source for AdV and for other infections. An interesting approach would be to apply the viral multiplex quantitative PCR assays, specifically designed for tracking sources of fecal contamination (Wolf *et al.* 2010).

To summarize, our results provide evidence that habitat overlap among humans, livestock, and wildlife can influence pathogen transmission ways and might facilitate especially the cross-species transmission of environmentally stable pathogens, such as AdV. This was clearly illustrated by the finding that livestock, companion animals and humans partially shed genetically highly similar AdV. The detection of HAdV in animals raises other questions to be investigated in future studies: Are animal carriers or intermediate hosts for HAdV and AdV from other hosts? Are HAdV still infectious if shed by animals? Which viral load in animal feces is infectious for humans?

Observed risk factors for the emergence of infectious diseases	Observed risk factors for zoonotic disease transmission
Demographic changes (e.g. immigration from neighbouring countries, civil war, increased population etc.)	**Slaughter, dissection and consumption of domestic animals and wildlife** (Contact with animal body fluid and blood)
Limited medical care and technology (deficient amount of health centres, shortage in medication, limited medical training, especially for emergency management)	**Close contact to domestic animals and wildlife** (free-ranging domestic animals, wildlife pets, habituated wildlife, etc.)
Commercial trends (wildlife hunt, bushmeat trade and consumption; rubber trade)	**Injury due to animals** (bitten by domestic animals or wildlife)
Climatic changes (change in the pattern of the rainy and dry season)	**Bad hygienic standards** (poor housing, consumption of non-treated water, well without cover, no running water, interrupted refrigeration chain, etc.)
Ecosystem disturbance (deforestation for rubber and cocoa plantation; hunting)	**No veterinary inspection** (no vaccination, vermifugation, lack of control of domestic animals etc.)
Bad health status (immunosuppression due to HIV-infection or other viral, bacterial or parasitical infection)	

Table 18. Observed risk factors for EID and zoonotic disease transmission

Based on Kuike, 2003: causes for the emergence of diseases (Kuiken *et al.* 2003)

Study limitations:

A study limitation was that the proportion of the total human (and animal) population sampled in each village is unknown, due to the unavailability of recent, comprehensive census data. Thus, one can not state that the study population is a representative subset of the local population and that the epidemiological data collected reflect the true situation in the region. The fact that more women than man volunteered to participate in the study and that in Taï only women working in the local restaurants were included show the unbalanced dataset of the study. The same applies for the animals, since unequal amounts of each species were sampled and since some animals were only sampled in few villages. For example, only few cattle were sampled, as cattle herds would leave the villages early in the morning in search of grazing pasture and because of the considerable effort required to safely collect the samples. The fact that only non-invasive samples were collected and analyzed partially limited the interpretation of our findings (e.g. shedding of HAdV by animals, pathogenicity of shed AdV). On the other side, analysis of rectum swabs and feces was an ethically correct and well-adapted approach for this first screening investigating the adenovirosphere in the study region. Potential temporal variations in pathogen exposure were not considered in this cross-sectional study design. Finally, the collection of more detailed, maybe even quantative data via interviews and questionnaires and over a longer period of time would have allowed a more exhaustive assessment of the risk factors for zoonotic disease transmission and for disease exposure.

Given the complexity of field missions conducted in remote, rural communities, this potential source of selection bias was unfortunately unavoidable.

6 Conclusion

This study highlights the benefits of a multidisciplinary One-Health approach to public and animal health. By combining medical and veterinary (virology, epidemiology, public health), environmental (ecology, biodiversity) and social (anthropology, politics) knowledge, it was attempted to obtain a more complete understanding of the risk factors for zoonotic EID in the study region. Furthermore, the findings revealed thus far unknown and fascinating aspects about the infection dynamics of AdV in animals and humans. Even if the pathogenicity of AdV is limited, these widespread viruses may represent a valuable tool to assess the risk for zoonotic and anthropozoonotic transmission of more pathogenic viruses in regions with high opportunity for animal-to-human exposure. The information gathered may be beneficial in formulating prevention recommendations to reduce pathogen transmission in tropical Africa.

Particularly in developing countries, where human, livestock and wildlife habitat overlap and that are considered to be hot-spots for EID, expansion of the One-Health concept is of paramount importance (Jones *et al.* 2008, Rabozzi *et al.* 2012). It has been shown that early detection and reporting of disease cases is essential for preventing global spread of epidemics. Essential for early reporting is knowledge on human and animal diseases, on pathogen transmission ways and on basic hygiene measures, also in remote areas (Halliday *et al.* 2012). Globalization favored the worldwide spread of several diseases (Gushulak *et al.* 2009), which originated in such isolated regions; the best known example being the meanwhile pandemic HIV (Hahn *et al.* 2000). But even on smaller scale, the transport of people, nutriments and livestock increase the risk for disease spread with epidemic or epizootic potential.

Large-scale public awareness and educational compaigns could fill the knowledge gaps and could provide information on feasible and acceptable preventive measures and alternatives. Moreover, more resources should be allocated to research on zoonotic EID in remote areas to investigate the complex multidirectional exchange of pathogens among humans, wildlife, and domestic animals. Furthermore, reinforcing the existing longitudinal global surveillance programs for human and animal diseases (e.g. Global Early Warning System, GLEWS and the Global Outbreak Alert and Response Network , GOARN**)** would facilitate rapid outbreak detection and public communication (Halliday *et al.* 2012). Different domestic (e.g. carnivores) and wild (e.g. chimpanzees) animal species could act as sentinels for health hazards (early warning systems) and could facilitate the disease surveillance (Cleaveland *et al.* 2006, Calvignac-Spencer *et al.* 2012).

In the Taï region, the strict implementation of basic hygiene practices (e.g. washing hands; cooking meat thoroughly; keeping animals away from cooking areas; burning sick animals) would already noticeably decrease the risk for zoonotic transmission. Additionaly, more fundamental changes (e.g. contruction of closed animal pens; continuous surveillance of wildlife; training of local animal health workers to provide veterinary, and preventive health services to the livestock; erection of field laboratories; vaccination campaigns) would considerably improve the life quality, by improving the health of both, humans and animals.

7 Summary: English and German Version

Summary: English version

Sub-Saharan Africa is considered to be a hotspot for emerging infectious diseases (EID) and the majority of these EID in humans originated from animal hosts and many are caused by viruses. In this first study on Adenovirus (AdV) in humans and domestic animals in Côte d`Ivoire, not only the prevalence and diversity of AdV shedding was assessed, but also the zoonotic and recombination potential of AdV was elucidated. The study region is situated next to the Taï National Park, the largest tropical forest of Western Africa. During two field missions in 2012, various samples were collected from the local population and from their domestic animals. Moreover the study participants were asked to fill out a questionnaire focusing on exposure to domestic animals and wildlife. Careful observations during the field missions, as well as the statistical analysis of the questionnaires revealed many risk factors for zoonotic disease emergence. Among others the following major drivers for zoonotic EID were identified: frequent contact to body fluids of domestic and wild animals (e.g. of non-human primates), poor hygiene standards (e.g. only limited access to drinking water and clean sanitary facilities), lack of veterinary inspections, insufficient medical care and demographic changes (massive immigration from neighboring countries). Hence we assumed that this region might be the ideal location to investigate whether and how human and animal habitat overlap influences rates and patterns of pathogen transmission between humans, livestock and wildlife.

AdV have been detected in mammals, birds, fishes, amphibians and reptiles, worldwide. Even though many AdV infections are asymptomatic in human and animal hosts, AdV-induced symptoms (e.g. gastroenteritis, kerato-konjunctivitis and pneumonitis) have been reported in different species. Bacterial co-infection, young age and immunosuppression enhance the risk to develop severe symptoms.

AdV prevalence and diversity in stool samples of humans and rectum/cloacal swabs of various animals (sheep, goat, cow, pig, dog, chickens and monkey) were determined with different PCR systems and subsequent sequencing. By phylogenetic and recombination analyses, the detected AdV strains were characterized, and their phylogenetic relation to recognized AdV types determined. Correlation between AdV infection and disease symptoms, and the effect of age and gender on infection status were analyzed statistically by descriptive statistics and by the application of generalized linear mixed models.

The prevalence of human AdV D (HAdV D) in human stool samples in the investigated sites was estimated to be 66 % in CI, 48 % in DR Congo, 28 % in Central African Republic (adults only) and 65 % in Uganda (adults only). Highly diverse HAdV D sequences were identified, among which a number are likely to stand for novel types. Younger individuals were more frequently infected than adults. There was no difference in HAdV D occurrence between genders. Moreover, no correlation was observed between HAdV D infection and clinical symptoms.

The overall prevalence of AdV shed was estimated to be 21.7 % for domestic mammals and 42.9 % for chickens. There was no difference in AdV occurrence between age groups. However, female animals were significantly more frequently infected than male animals. Highly diverse and potentially novel AdV apparently circulate among the animal population: strains from 3 different AdV genera

(*Mastadenovirus, Aviadenovirus* and *Siadenovirus*) were identified and intriguingly HAdV were repeatedly detected in animal rectum swabs. On one hand animals shedding various AdV (including HAdV) can be considered to be reservoir or mixing vessels; on the other hand shedding might only be due to the ingestion of contaminated material and following passive passage. However, it can be assumed that this shedding might play an important role for virus spread and probably for human and animal health. Moreover, potential pathogenicity, modes of transmission, and sources in nature were discussed.

To conclude, AdV were detected with a high prevalence and diversity in the human and animal study populations. Further investigations are needed to pinpoint pathological potential of each of the identified viruses. The study findings revealed evidence for anthropozoonotic (human-to-animal) and cross-species transmission of AdV and for recombination. Although no zoonotic transmission (animal-to-human) of AdV was detected, the observed multitude of risk factors for zoonotic transmission certainly favors the cross-species transmission of other pathogens and underlines the importance of research in this potential hotspot for EID. The information gathered may be beneficial in formulating prevention recommendations to reduce pathogen transmission in areas where humans, livestock and wildlife cohabit.

Zusammenfassung: deutsche Version

Das sub-saharische Afrika gilt als ein Hotspot für neuartige Infektionskrankheiten (emerging infectious diseases, EID), und die Mehrheit dieser EID beim Menschen stammten aus tierischen Wirten und wird zu einem überwiegenden Teil durch Viren verursacht. In dieser ersten Studie über Adenoviren (AdV) bei Menschen und Haustieren in Côte d `Ivoire wurde nicht nur die Verbreitung und Vielfalt der AdV-Ausscheidung beurteilt, sondern auch deren zoonotisches und Rekombinations-Potenzial aufgeklärt. Das Untersuchungsgebiet befindet sich neben dem Taï-Nationalpark, dem größten tropischen Regenwald in Westafrika. Während zwei Feldmissionen im Jahr 2012, wurden verschiedene Proben von der lokalen Bevölkerung und von ihren Haustieren gesammelt. Darüber hinaus wurden die Studienteilnehmer gebeten, einen Fragebogen mit speziellem Fokus auf die Exposition zu Haus- und Wildtieren auszufüllen. Sorgfältige Beobachtungen während der Feldmissionen sowie die statistische Auswertung der Fragebögen bestätigten das Vorkommen vieler Risikofaktoren für die Entstehung von Zoonosen. Unter anderem wurden die folgenden wesentlichen begünstigenden Faktoren für zoonotische EID identifiziert: häufiger Kontakt zu Körperflüssigkeiten von Haus- und Wildtieren (z.B. von nicht-menschlichen Primaten), schlechte Hygienestandards (z.B. nur begrenzter Zugang zu sauberem Trinkwasser und sanitären Anlagen), keine tierärztliche Überwachung und unzureichende medizinische Versorgung, demografischer Wandel (z.B. massive Einwanderung aus den Nachbarländern) etc. Damit bietet diese Region ideale Studienbedingungen, um zu untersuuchen, wie und ob das Überschneiden von menschlichen und tierischen Lebensräumen Ausmaß und Muster der Übertragung von Krankheitserregern zwischen Mensch und Tier beeinflusst.

AdV wurden in Säugetieren, Vögel, Fischen, Amphibien und Reptilien auf der ganzen Welt identifiziert. Auch wenn viele AdV-Infektionen asymptomatisch verlaufen, wurden sowohl im humanen, als auch im tierischen Wirt AdV-induzierte Symptome (z.B. Gastroenteritis, Kerato-konjunctivitis und Pneumonie) nachgewiesen. Bakterielle Ko-Infektion, junges Alter oder Immunsuppression erhöhen das Risiko für schwere Symptome. In letzter Zeit, wurde mehrmals von zoonotischer Übertragung, sowie von Rekombination zwischen verschiedenen AdV berichtet.

AdV Prävalenz und Vielfalt wurden in Stuhl-Proben von Menschen und in rektalen/kloakalen Abstrichen von verschiedenen Tieren (Schaf, Ziege, Kuh, Schwein, Hund, Huhn und Affe) mit diversen PCR-Systemen und anschließender Sequenzierung bestimmt. Durch phylogenetische und Rekombinations-Analysen wurden die isolierten AdV-Stämme charakterisiert und ihre phylogenetischen Verhältnisse zu anerkannten AdV-Typen bestimmt. Die Korrelation zwischen Adenovirus-Infektion und Krankheitssymptomen, Prävalenz-Unterschied und der Einfluss von Alter als auch Geschlecht auf Infektionsstatus wurde anhand verschiedener statistischer Modelle berechnet.

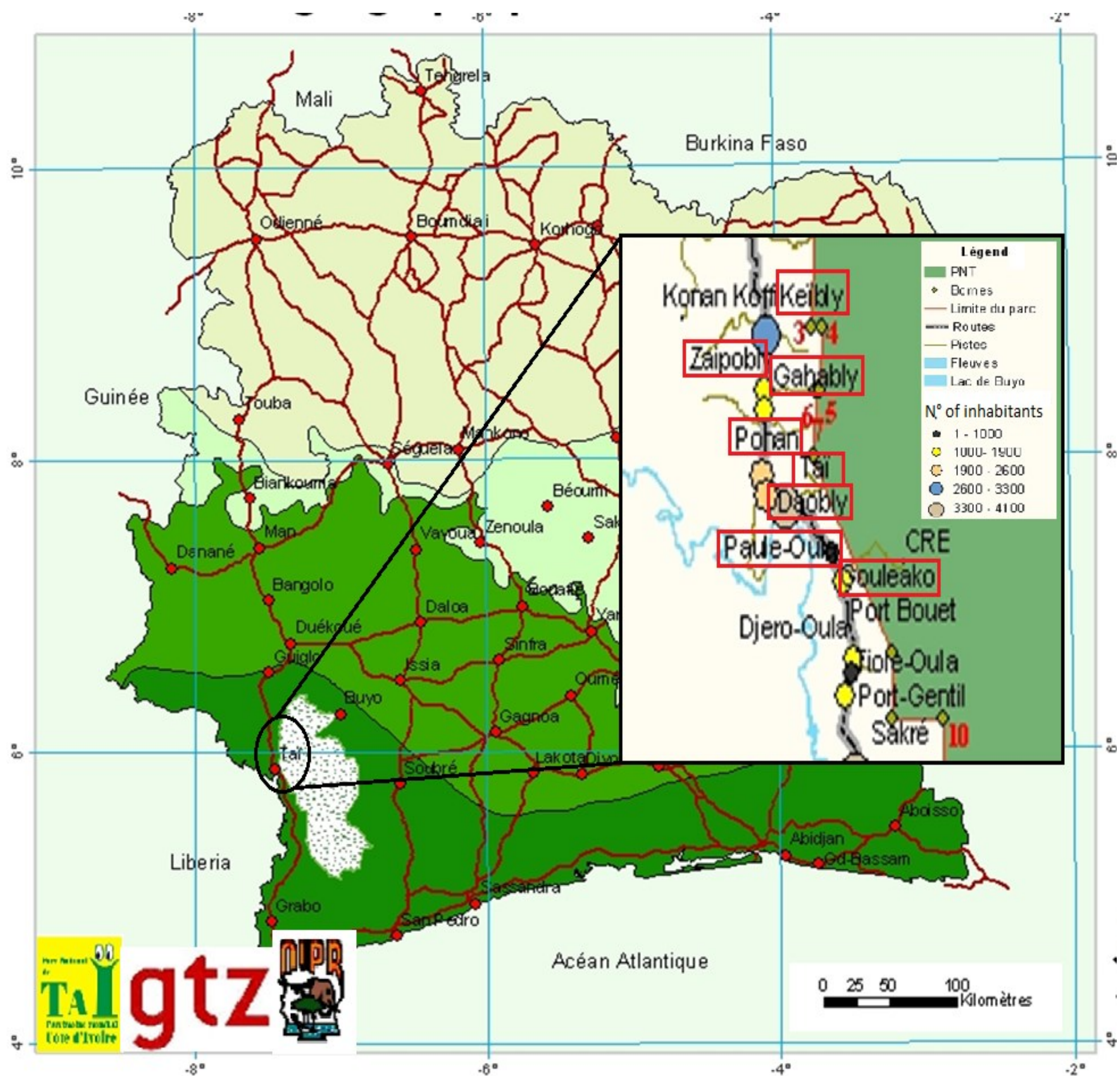
Die Prävalenz von humanen AdV D (HAdV D) in den menschlichen Stuhlproben war 66 % in CI, 48 % in der DR Kongo, 28 % in der Zentralafrikanischen Republik (nur für Erwachsene) und 65 % in Uganda (nur für Erwachsene). Unterschiedliche HAdV D-Sequenzen wurden identifiziert, von denen eine Reihe neuartige Typen darstellen könnten. Jüngere Personen waren häufiger infiziert als Erwachsene; es gab keine geschlechtsspezifischen Unterschiede im HAdV D Auftreten. Es wurde kein Zusammenhang zwischen HAdV D-Infektion und klinischen Symptomen gefunden.

Die Analysen bei Tieren ergaben eine Gesamtprävalenz von AdV in Haustieren von 21.7 % und in Hühner von 42.9 %. Es gab keinen Unterschied in AdV-Auftreten zwischen den Altersgruppen, aber weibliche Tiere waren signifikant häufiger infiziert als männliche Tiere. Eine große Vielfalt an

potenziell neuartigen AdV scheint in der Tierpopulation zu zirkulieren: Typen aus 3 verschiedenen AdV Genera (*Mastadenovirus, Aviadenovirus* und *Siadenovirus*) wurden identifiziert und interessanterweise wurden HAdV auch von tierischen Rektal-Tupfern isoliert. Einerseits, ist es gut möglich, dass Tiere, die verschiedenste AdV (einschließlich HAdV) ausscheiden, als Reservoir oder als „mixing vessel" für AdV dienen; andererseits aber kann das Auscheiden nur bedingt sein durch die Einnahme von kontaminiertem Material und folgendem passivem gastrointestinalen Durchgang. Allerdings kann man davon ausgehen, dass die AdV-Ausscheidung eine wichtige Rolle für die Verbreitung der Viren und wahrscheinlich auch für die menschliche und tierische Gesundheit spielt. Darüber hinaus, wurden potenzielle Pathogenität, Übertragungswege und natürliche Quellen des Virus diskutiert.

Abschließend kann gesagt werden, dass AdV mit einer hohen Prävalenz und Diversität in den menschlichen sowie tierischen Studienpopulationen nachgewiesen wurden. Weitere Untersuchungen sind erforderlich, um das pathologische Potential jedes dieser Viren festzustellen. Die Ergebnisse dieser Dokotorarbeit ergaben deutliche Anzeichen für anthropozoonotische (Mensch-zu-Tier) und Interspezies-Übertragung von AdV und für Rekombinationsereignisse. Obwohl keine zoonotische (Tier-zu-Mensch) Übertragung von AdV identifiziert wurde, begünstigt die beobachtete Vielzahl von Risikofaktoren für zoonotische Übertragung sicherlich die Inter-spezies Übertragung anderer Krankheitserreger und unterstreicht die Bedeutung der Forschung in diesem potenziellen Hotspot für EID. Die gesammelten Informationen könnten nützlich sein bei der Formulierung von Empfehlungen für die Prävention von Erreger-Übertragung in Regionen, in denen Menschen, Haustiere und Wildtiere eng zusammenleben.

8 Supplementary files



Supplementary Figure 1. Map of Côte d`Ivoire and sampling region

NUMERO ID **NOM** **DATE** **VILLAGE**

ANIMAUX	1x/j.	1x/s.	1x/m.	Occasions spéciales	pas mangé adulte	pas mangé enf. F	pas mangé enf. M	Raison	vous chassez et comment?	dépecez vous?	préparez-vous la viande	État de la viande	Prov.	Commentaires
Volaille(poules)														
Moutons														
Chèvres														
Porcs														
Vache														
Poissons														
Singes														
Chimpanzee														
Antelope (Biche)														
Porcs sauvages														
Ronguers														
Escargots														
Chauves Souris														
Cyvette														

VEGETALE	1x/ j.	1x/s.	1x/m.	Occasions spéciales	Jamais	Provenance	Raisons	Utilisation
Céréales / Tubercles								
Légumes								
Légumes de la forêt								
Fruits								
Huile local								
Boissons alc.								
Plantes méd. 1								
Plantes méd. 2								
Plantes méd. 3								

Raison pas m 1. Coutûme 2.Personnel 3.Allergie 4.Autres

État de la vian 1.fraîche 2. fumée 3. autres

Provenance 1. Champs 2. pers. privée 3. chasse 4. pêche 5. élevage 6. marché 7.Forêt

Supplementary Figure 2.Part 1 of the questionnaire

1. Où préparez-vous la nourriture (la viande)?

2. Qui prépare la nourriture pour vous et assiste pendant la preparation?

3. Est-ce qu'il y a des animaux/organes qui sont mangés crus?

4. Y-a-t`il des parties d`animaux/des organes/ des fluides corporelles que vous ne mangez jamais?

> Intestins > lésions cutanées > autres lésions > autres:
> cerveau > sang > organes génitaux

5. Quels autres produits animaux mangez-vous?

> lait > miel
> graisse animale > autres

6. La viande de quels animaux mangez-vous le plus souvent?

Rang de 1 à 3 (1 mangé le plus souvent, 3 mangez le moins)

1. 2. 3.

7. Pendant quels activités entrez-vous en contact direct avec des singes?

>pendant l`abattage >en chassant >contact avec animal de compagnie
>en mangeant >en cuisina >autres:

8. Est-ce que d`autres membres de la famille sont en contact avec des animaux?

Genre ? Si oui
>Qui:
>Genre de contact

9. Avez-vous déjà été mordu ou blessé par un ?
animal ? Si oui
> Animal responsable: > Thérapie:

> Cicatrices: >Photos

10.Est-ce que vous avez un animal qui représente pour vous votre Totem personnel?
Si oui, lequel

11. Quels animaux possédez-/élevez-vous?

>bovins >chèvres >moutons
>volaille >chien >autres:

Pour propriétaire d`animaux:

12. Est-ce que vos animaux ont déjà été traités par un vétérinaire?
Si oui, pour quel traitement
> Vermifuge >Vaccin >Autres

13. Est-ce que vous avez remarqué un nombre inhabituel de morts chez vos animaux ? Si oui, chez quels animaux:

14. Avez-vous remarqué des symptomes spécifiques avant/pendant la mort?

> comportement bizarre >lésions
> symptomes respiratoires >symptomes gastroentérologiques

15. Que faites-vous des cadavres ?

> incinérés >enterrés >autres
> utilisés comme nourriture pour les animaux
>utilisés comme nourriture pour les humains

Supplementary Figure 2. Part 2 of the questionnaire

Country	Setting	Hospitalized	Age (years)	Symptoms	Sample type	Samples tested	Test	HAdV-positive samples (%)	SequencedHAdV-positive samples	HAdV D positive samples (%)	Ref.
Bangladesh	urban	?	children (0-3)	gastroenteritis	stool	917	PCR	17 (2)	17	10 (1.1)	(Dey *et al.* 2009)
Thailand	urban	yes	children (0-15)	gastroenteritis	stool	1138	PCR	69 (6)	76	2 (0.2)	(Sriwanna *et al.* 2013)
			adults (>15)			124		2 (2.6)			
Botswana	urban	?	children (0-5)	gastroenteritis	stool	346	ELISA	27 (8)	n.d	n.d.	(Basu *et al.* 2003)
Brazil	urban	yes	children (0-5)	gastroenteritis	stool	3060	EIA	61 (2) (EIA)	59	3 (0.09)	(Filho *et al.* 2007)
Burkina Faso	urban	yes	children (0-5)	gastroenteritis	stool	66	Microscopy and immunochromatography	1 (1.5)	n.d	n.d.	(Djeneba *et al.* 2007)
	urban	yes	Adults and children (?)	gastroenteritis	stool, urine, respiratory	2300	qPCR, PCR	92 (4)	57 (randomly chosen)	1 (0.07)	(Berciaud *et al.* 2012)
Germany	urban	yes	children (?)	gastroenteritis	stool	129	PCR	31 (24)	31	0 (0)	(Oh *et al.* 2003)
		no		none	stool	28		3 (14)	3	0 (0)	
Kenya	urban	yes	children (0-14)	gastroenteritis	stool	137	PCR	60 (43.8)	55	18 (13.1)	(Magwalivha *et al.* 2010)
	rural					80		34 (42.5)	16	3 (3.8)	
	urban			none		61		10 (16.4)	7	1 (1.6)	
	rural					n.d.		n.d.		n.d.	
Ghana	peri-urban	yes	children (0-11)	gastroenteritis	stool	367	PCR	73 (19.9)	73	23 (6.3)	(Silva *et al.* 2008)
Nigeria	urban	yes	children (0-5)	gastroenteritis	stool	138	Cell Culture and EIA	23 (17)	n.d	n.d	(Audu *et al.* 2002)
				none		29		4 (14)			
Tunisia	?	yes	children (0-5)	gastroenteritis	stool	638	EIA and ELISA	35 (6)	n.d	n.d	(Fodha *et al.* 2007)
France	urban	yes	children (0-4)	gastroenteritis	stool	973	EIA	49 (5)	n.d	n.d	(Tran *et al.* 2010)
Japan	urban	no	children (?)	gastroenteritis	stool	88	PCR	11 (12.5)	n.d.	n.d.	(Akihara *et al.* 2005)
				none		833		96 (41.7)			

Supplementary Table 1.Published studies on adenovirus prevalence in children with gastroenteritis

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