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EBOLA IN GREAT APES - CURRENT KNOWLEDGE, POSSIBILITIES FOR VACCINATION AND THE IMPLICATIONS FOR CONSERVATION AND HUMAN HEALTH

ABSTRACT

1. Ebola virus disease (EVD) is a threat to human health and the survival of African great apes. The disease has led to major population declines of chimpanzees and gorillas, and infected great apes play an important role as sources of human EVD outbreaks. The threat posed by EVD raises the question whether vaccination of wild apes is a possible strategy to reduce the occurence and impact of this disease.

2. This article reviews the current knowledge about EVD in great apes and emphasizes the link between ape and human outbreaks. It discusses the need for control strategies such as vaccination and describes aspects of primate behavior, virus biology, vaccine composition, and vaccination principles that are necessary to consider when making management decisions about great ape vaccination. Finally, it identifies gaps in the understanding of Ebola ecology and highlights surveillance and research that can aid the survival of great apes and reduce human exposure to Ebola virus.

3. The unpredictable emergence of Ebola viruses and the severe impact of EVD call for efficient monitoring and ultimately control of Ebola. This article provides a platform for further interdiciplinary discussions to decide on optimal management solutions regarding vaccination of great apes against Ebola.

KEY WORDS: conservation, Ebola, global health, vaccination, wild great apes

RUNNING HEAD: Ebola in great apes

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INTRODUCTION

Ebola virus disease (EVD) is an acute, severe and lethal disease of particular concern for humans, chimpanzees, and gorillas (Karesh & Reed 2005, Feldmann & Geisbert 2011). The disease is caused by viruses of the Ebola genus, belonging to the virus family Filoviridae (Kuhn *et al.* 2010). The Ebola genus comprises five distinct species, although they are generally referred to as the Ebola virus or simply Ebola. Since the first Ebola virus was discovered in 1976, Ebola viruses (EVs) have sporadically re-emerged from an unknown reservoir and caused more than 20 human outbreaks across Africa (Anonymous 2015a). The epidemic that started in West Africa in December 2013 has lasted for more than two years and claimed more than 11,000 lives (Anonymous 2015b).

EVD is also considered a major threat to the survival of African great apes, all of which are currently listed as Endangered or Critically Endangered (Anonymous 2016a). The disease in apes is also of concern for human health as there is a clear link between Ebola-infected apes and human EVD outbreaks. For all eight human outbreaks in Gabon and the Republic of Congo (ROC) which have occurred in the last 25 years, there is epidemiological evidence (and in three of these outbreaks also laboratory evidence) that they were initiated by contact to infected bush meat from gorillas (*Gorilla gorilla gorilla*) or chimpanzees (*Pan troglodytes troglodytes*) (Georges *et al.* 1999, Rouquet *et al.* 2005), hence hunting or scavenging of wild great apes is a major risk factor. Bats, monkeys, and perhaps other wildlife are also possibly able to infect humans; however, the true reservoir(s) and natural circulation of EVs still remain unidentified (Lahm *et al.* 2007, Leendertz *et al.* 2015, Maganga *et al.* 2014, Mari Saez *et al.* 2015, Olival & Hayman 2014, Olson *et al.* 2012). This makes prediction, surveillance and control of EVD challenging.

The threats posed by EVD, raise the question of whether vaccination of wild great apes would be a future option to help mitigate the effect of EVD on ape populations and protect human health. Although no outbreak has been confirmed in apes since 2005, the unpredictable pattern of virus emergence and the severe impact of outbreaks mean that there is a true concern of future occurrences.

This article provides an overview of what is known about EVD in great apes, and highlights surveillance and research that can aid the survival of wild great apes and reduce human exposure to Ebola virus. Moreover, it describes aspects of primate behavior, Ebola virus biology and ecology, vaccine composition, and vaccination principles that are essential to know when considering vaccination of great apes. This article provides a platform for further interdiciplinary discussions to decide on optimal management solutions regarding protection of wild great apes against Ebola.

EBOLA VIRUS DISEASE

Four separate species of EVs cause EVD in Africa; *Zaire EV (*ZEBOV*), Sudan EV (*SEBOV*), Cote d'Ivoire EV (*TFEBOV) *and Bundibugyo EV (*BEBOV), of which ZEBOV and TFEBOV have so far been detected in great apes (Leroy *et al.* 2004, Rouquet *et al.* 2005, Wittmann *et al.* 2007, Kuhn *et al.* 2010). The genetic diversity of these Ebola species represents a hurdle for vaccine development since a vaccine working against one species might not protect against another (i.e. lack of cross reaction) (Feldmann & Geisbert 2011). A fifth member of the Ebola genus (*Reston Ebola virus*, REBOV) exists in Asia; however, this virus is not thought to be lethal to humans, hence the EVD pathogenesis described below does not apply to this virus species. There have been no reported cases of EVD in orang-utans.

The biological aspects of the EVD disease process are similar in humans and apes (Kuhn 2008). EVs are highly infectious and enter the body through contact with mucus membranes, eyes, and broken skin. After an incubation period of 2-21 days, the infected individual develops symptoms which include fever, vomiting, diarrhoea, internal and sometimes external bleeding. There are few observations of wild apes that have presumably been suffering from EVD, but signs of abdominal pain, lethargy, poor appetite, diarrhoea and emaciation or simply “abnormal behaviour” have been noted in great apes, and bleeding from the nostrils has been observed among other non-human primates (NHPs) (Formenty *et al.* 1999, Georges *et al.* 1999, Lahm *et al.* 2007). Other individuals can get infected through contact with the body or body fluids from a sick or dead member of the social group (Warren & Williamson 2004). It is debated how infection can spread through ape populations over large geographical areas and there are two main theories. The first theory proposes that the spread occurs solely by group-to-group infection after a single or few introductions of the virus from the original reservoir or other infected animals into an uninfected ape population. Such spread could occur via direct contact such as intergroup encounters, extra-community copulation, and emigration of females (including infected gorilla females leaving the group after death of the silverback), or indirectly, via sharing of feeding sites or home range (Vigilant *et al.* 2001, Bermejo 2004, Bradley *et al.* 2004). EV has been isolated in semen of infected human males many months after infection (Chughtai *et al.* 2016) indicating that surviving male apes could initiate new infection chains if mating occurs within the infectious period. The potential for group-to-group spread in apes depends on several factors including the incubation time of the specific individual (or longevity of infectious semen), travel distance and ape density (Walsh *et al.* 2007, Walsh *et al.* 2009, Ryan *et al.* 2013). The sociality of African apes, including the natural fission-fusion social system of chimpanzees (Lehmann & Boesch 2004) will also influence the spread of virus in a positive or negative way, depending on the contact pattern and which individuals are infected.

The second theory of how infection can spread over large areas proposes that outbreaks occur mainly as a direct consequence of repeated spill-over from a so far unidentified natural host into geographically separated ape social groups. The outbreak distribution is consequently defined by the dispersal and movements of the reservoir host itself rather than the apes (Leroy *et al.* 2004). However, these two theories are not mutually exclusive, as it is possible that both these modes of transmission are important, and that one or the other can predominate at times or sites depending on the underlying factors that trigger or sustain the outbreaks. In any case, Ebola's tremendous ability to spread rapidly once it has emerged is illustrated by the recent human outbreak in West Africa, which started with one infected child and subsequently included nearly 30,000 people (Mari Saez *et al.* 2015, Anonymous 2015c).

Experimental infections show that deaths in NHPs occur within a similar time frame as in humans (ca.8-10 days after onset of symptoms for ZEBOV and ca.12-14 days for TFEBOV) (Geisbert *et al.* 2009). Although the mortality rate can be extremely high (up to 88% in humans and possibly higher in great apes infected with ZEBOV [Bermejo *et al.* 2006, Anonymous 2015c]), it is evident that recovery and survival after infection is possible. Antibodies to Ebola virus have been detected in blood from humans, wild-born chimpanzees and other primates and in the feces from wild-living gorillas, proving that some individuals exposed to the virus can mount an immune response and recover after infection (Leroy *et al.* 2004, Lahm *et al.* 2007, Reed *et al.* 2014). Survival of individuals in great ape groups whose other members have died of Ebola also suggest that recovery is possible (Formenty *et al.* 1999, Bermejo *et al.* 2006); however, without laboratory testing of samples, it is not possible to ascertain whether these individuals were in fact infected or managed to escape exposure. The infectious, acute and often lethal nature of Ebola shows, however, that great apes are unlikely to be able to indefinitely sustain an infection chain.

An outbreak stops naturally when no more susceptible individuals come in contact with infectious apes (dead or alive) or infectious materials. Leroy and colleagues (2004) reported carcasses remaining infectious in the rainforest for 3-4 days; it remains however uncertain how long EV survives under different environmental conditions (Anonymous 2014a).

OCCURENCE AND IMPACT OF EVD IN GREAT APES

EVD in great apes was first recognized nearly twenty years after the Ebola virus was discovered in humans (Pigott *et al.* 2014). Ape outbreaks have mainly been detected in Central Africa whereas human outbreaks have occurred across tropical Africa (Figure 1).

TFEBOV was the first ape-associated Ebola species to be discovered when 12 habituated chimpanzees (*Pan troglodytes verus*) in the Taï National Park, Côte d'Ivoire, died or disappeared within a few weeks in November 1994 (Formenty *et al.* 1999). Ebola was confirmed by immunohistology staining of virus in carcass samples and by virus isolation from a researcher who contracted EVD after exposure to the carcass. Mortality rate in the chimpanzee group was estimated to ca. 25%, similar to what has been observed in outbreaks of human BEBOV (an Ebola species not detected in apes) (Towner *et al.* 2008). There was no further human-to-human transmission in this case and TFEBOV has not been detected again in Africa.

The ZEBOV species has had a much more significant impact on great apes. Outbreaks have occurred in North Eastern Gabon and ROC in two clusters: first in the mid-1990s and then in the first five years of this century. Surveillance of wild great apes is challenging and does not cover all wild populations hence outbreaks could also have occurred undetected at other times. Most of what is known about the outbreaks in apes in North Eastern Gabon in the mid-1990s emanated from investigations of three human outbreaks that occurred at that time (Georges *et al.* 1999). In November 1994, there were concurrent reports of wildlife deaths among the local gorilla and chimpanzee populations when the first human cases appeared in gold mining camps in the Minkebé forest area. About a year later, butchering a dead chimpanzee found ca. 40 km further south initiated a second human outbreak. The third human outbreak started yet another 160 km further south six months later with the death of two hunters, of which one was said to have killed several mangabeys in the time before he got sick. Dead chimpanzees and gorillas were encountered on several occasions during and after these outbreak years, indicating that EVD was affecting the apes for a long time (Georges *et al.* 1999, Lahm *et al.* 2007). From these dead apes, a skin sample tested positive in Ebola immunohistology staining.

Wildlife surveys carried out in Minkebé and Mwagne forest blocks before and after these outbreak years showed a dramatic reduction (90-98%) in the gorilla and chimpanzee populations that could have been caused by a highly lethal, rapidly spreading disease like EVD (Huijbregts *et al.* 2003, Maisels *et al.* 2004, Lahm *et al.* 2007). In comparison, the mortality in humans exposed to the same virus was 59-75% in the concurrent outbreaks. The relatively lower human mortality is possibly due to hospital care of infected people rather than a higher biological disease resistance.

A surveillance network was subsequently set up in North Eastern Gabon and ROC due to the association between human EVD outbreaks, the regularity of human contact with apes and/or ape meat in these countries and the decline of great ape populations. This network, which also included monitoring and sampling of a range of wildlife carcasses, revealed that five human EVD outbreaks that occurred between October 2001 and May 2005 resulted from contact to infected carcasses, mainly gorillas and chimpanzees (Leroy *et al.* 2004, Rouquet *et al.* 2005, Nkoghe *et al.* 2011). Ebola positive ape carcasses were found in several forest areas including Lossi and Odzala National Parks, ROC, where population losses exceeding 90% were observed in habituated gorillas (Bermejo *et al.* 2006). In Lossi alone Ebola was estimated to have killed 5,000 individuals. Ebola is believed to have caused ape deaths in Odzala until at least 2007 (Caillaud *et al.* 2006, Cameron *et al.* 2016), however, since 2005, no further cases of EVD in African apes have been confirmed. A human EVD outbreak in Boende, DRC, in 2014 might have been linked to contact with a primate carcass (species unidentified) (Maganga *et al.* 2014), however, no samples have been obtained for testing and the link remains speculative. The human 2013-2016 EVD epidemic in West Africa is not believed to be associated with deaths of great apes or other primates, but is rather thought to be the consequence of a child’s accidental contact with a large colony of insectivorous bats (Mari Saez *et al.* 2014). The area where the epidemic started harbours few primates, no case of dead apes has been reported and recent surveys indicate no decline in primate populations compared with surveys conducted prior to the outbreak.

In summary, EVD has likely killed thousands of gorillas and chimpanzees in the central African rainforests where the majority of the world’s remaining great ape populations reside (Vogel 2006, Hopkin 2007). Because of these losses, the IUCN upgraded the western lowland gorilla to critically endangered in 2007 (Walsh *et al.* 2007). Carcass samples and precise assessments of wild great ape population sizes are challenging to obtain, making it difficult to estimate the true impact of Ebola on great apes. There is, however, no reason to doubt that EVD can severely affect great ape populations; and in fact, until the start of the recent epidemic in West Africa, the number of apes estimated to have succumbed to Ebola was far greater than the total number of people (ca.1500) who died from the same virus in all known human outbreaks (Anonymous 2015b).

WHERE AND HOW DO THE APES GET INFECTED WITH EBOLA?

It is evident from the disease’s rapid spread and high mortality in great apes that these primates are neither natural, asymptomatic carriers of the virus nor able to sustain the infection indefinitely, which are both important criteria for a reservoir host (Olival & Hayman 2014). The apes are rather so-called ‘accidental hosts’ who act as a source of infection for human outbreaks when they themselves are infected from another host(s) sharing the same habitat. Numerous carcasses of other animals found in the Gabon and ROC forests between 1994 and 2005 indicate that many species may be susceptible to EV, although the virus has only been isolated from gorillas, chimpanzees and a duiker (*Cephalophus* sp.) despite intensive testing of wildlife carcasses in outbreak areas for decades (Leroy *et al.* 2004, Lahm *et al.* 2007, Wittmann *et al.* 2007). Laboratory analyses of samples from certain fruit eating and insect eating bats indicate that these bats were able to host, possibly spread and indeed survive infection; however, the virus itself has never been isolated from these hosts and more research is necessary to confirm that the virus is circulating primarily in any of these species or if yet another host represents the true reservoir (Olival & Hayman 2014, Leendertz *et al.* 2015). Until the natural history and ecology of Ebola are more fully characterized, it is only possible to speculate about if and how apes (and humans) get infected from bats or other species (Viana *et al.* 2014). Apes are not known to hunt bats, but could potentially come in direct contact with fruit bats roosting in trees where apes feed, or with insectivorous bats roosting in tree holes where the apes search for honey, insects or water (Nishida 2010). Alternatively they could get infected via excretions from any kind of bat or other animal when ingesting contaminated food via feces, urine or saliva, although such excretion of EV in naturally infected bats is yet to be confirmed (Olival & Hayman 2014). Direct contact with other infected hosts is another possible infection route, including hunting of monkeys or other prey that might be infected and/or during inspections of infected carcasses (Boesch & Boesch-Achermann 2000, Karesh *et al.* 2012). In the chimpanzee outbreak in Côte d'Ivoire, red colobus monkey predation was considered a risk factor (Formenty *et al.* 1999), but was not confirmed as a transmission route.

The uncertainties of Ebola ecology make predicting outbreaks in wildlife (and humans when no previous wildlife outbreak is detected) nearly impossible and without further data most of the tropical belt of Africa is considered a risk area for Ebola emergence (Pigott *et al.* 2014). This has implications for outbreak preparedness in humans and wildlife alike. What is clear, however, is that apes are intermittently exposed to EVs in their natural environment, which means that we might not be able to remove, or prevent exposure to, the source of infection. This highlights the need for considering strategies to prevent EV infection of great apes, and to also break the link between them and human EVD outbreaks (Feldmann & Geisbert 2011).

THE BENEFITS AND POSSIBILITIES OF PREVENTING OR REDUCING SPREAD OF EBOLA WITHIN APE POPULATIONS

Considering the severe reduction in population number and dissemination of groups that follows EVD outbreaks in great apes it is highly questionable if leaving this disease to be regulated by nature itself is a viable option. Demographic structure changes (for example increased frequency of emigration to start new breeding groups) of populations after severe declines can indeed occur to favour population growth (Genton *et al.* 2012, Genton *et al.* 2014, Reed *et al.* 2014), however, in the case of repeated outbreaks and added pressure from other infectious diseases, bushmeat hunting and deforestation these recovery strategies might not be sufficient to prevent extinction (Leendertz *et al.* 2006, Morgan & Sanz 2007, Kondgen *et al.* 2008, N'Goran *et al.* 2012). Apes have a relatively long generation time (Anonymous 2014a) which means that an outbreak of a disease with high mortality rate such as EVD requires a very long recovery time for affected populations; some calculated that it would take the affected gorilla population in Lossi more than 130 years to recover to initial population numbers (Ryan & Walsh 2011). In addition, fragmentation of populations due to mortality also has a negative impact on the genetic diversity of the populations, which could exacerbate extinction risk especially when populations are reduced in size. Therefore, it is clear that efficient strategies to reduce the impact on EVD in wild population will be beneficial for conservation. Further, prevention or reduction of infectious diseases in wildlife, including preservation of natural ecosystem integrity and biodiversity, will ultimately have a positive impact on human health (Rabinowitz *et al.* 2013). It should however be noted that not all human EVD originated from infected great apes, hence such strategies will not prevent human exposure to EVs from other wildlife sources (Anonymous 2015a).

The questions that arise when any wildlife is concerned, is if any intervention in a natural ecosystem is justified. The benefits of any intervention must be assessed in conjunction with the potential negative impacts (Fedigan 2010, Gruen *et al.* 2013). Nearly all great ape populations have been, and continue to be, impacted by human influence, such that apart from some of the protected areas, the majority of the African forests can no longer be considered 'natural' (Laurance 2009, Hockings *et al.* 2015). Preserving protected species is required by law and there should be an additional moral responsibility to preserve endangered species, and further, human-induced environmental changes such as deforestation and urbanisation may exacerbate the likelihood of EV emergence (Waldman 2015). Hence it seems rational to focus upon how to protect the remaining wild great apes from a disease that are incompatible with survival of the species should outbreaks continue to occur, rather than discussing if human intervention is justified. Hypothetical possibilities to prevent the spread of Ebola within ape populations exist, however the questions of which strategy or strategies would be feasible and efficient, despite limited knowledge about the natural ecology of these viruses and their unpredictable emergence, remains.

In human EVD outbreaks, isolation of infected cases remains an important control strategy (Anonymous 2015d). Protection of the great apes cannot rely upon similar isolation strategies, although it might naturally occur to some degree as apes sometimes temporarily leave the rest of the social group when experiencing signs of disease (Boesch & Boesch-Achermann 2000). It is evident that some isolated groups of chimpanzees and gorillas continued to thrive in one instance only 20km from an outbreak area (Lahm *et al.* 2007), hence geographical separation could theoretically provide protection. However, attempting to isolate social groups by cutting corridors in the forest in order to stop spread of infected apes is unlikely to be feasible, desirable or efficient for various reasons. Firstly, clearing of larger geographical areas in dense and remote rainforest areas is laboursome and in conflict with forest preservation, and as apes are able to cross large open areas the effort might not even be worthwhile (Haurez *et* *al.* 2013). Further, should separation be successful it might create stress for the apes due to man-made disturbance and change in their territory, and in longer term compromise the population genetic viability. And finally, the virus could be introduced to other parts of the forest by reservoir hosts not restricted by human-made barriers, which make attempts to geographically separate ape groups to avoid spread of infection redundant.

Preliminary results regarding experimental post-exposure treatments based on protective antibodies (e.g. ZMapp) are encouraging; however, patients require additional intensive care, hence such medication is unlikely to be effective in controlling EVD outbreaks in wild apes, even when habituated. Vaccination, i.e. administration of preventative medication which stimulates an immune response that will protect the individuals against natural exposure to the virus, could possibly be the most promising control strategy in great apes. Indeed, the prospect of safe and efficient Ebola vaccines in the near future invites a discussion about their use in wild populations of great apes (Marzi & Feldmann 2014). There are significant ethical issues when considering the vaccination of wild great apes, though it should be noted that in humans the prospective use of vaccines is also not without its own ethical and practical concerns (Cohen & Kupferschmidt 2014, Lee *et al.* 2015).

It is not the first time that vaccines have been used in wild apes (Ryan & Walsh 2011). For example, small scale vaccination has been done in habituated (or reintroduced) populations for diseases like measles, poliomyelitis, tetanus, and anthrax (Goodall 1986, Hastings *et al.* 1991, Ryan & Walsh 2011). Vaccinations were done through distance darting, injections when other interventions were undertaken under general anaesthesia, or in the case of chimpanzee poliomyelitis in the mid-1960s, through feeding of bananas; an acceptable practice at the time.

THE PROSPECT OF VACCINATING WILD GREAT APES AGAINST EBOLA

Implementation of great ape vaccination programs seems superficially straightforward, since humans and great apes are genetically and physiologically similar and exhibit similar immunological reactions to vaccines. However, accessibility of great apes due to their elusive nature and occurrence in remote habitats, limits the choice of vaccine(s) and call for well-considered vaccination strategies. A thorough understanding of EVD, the great apes natural behavior, and the vaccines that might become available in the future is required for discussing and deciding on appropriate vaccination strategies.

CURRENT VACCINE CANDIDATES

In the last decades, a number of vaccine types have been tested in suitable animal models including non-human primates and some have subsequently advanced into human clinical trials (Marzi & Feldmann 2014, Falzarano 2016). Regardless if a vaccine is intended for human or great ape use, it is extremely important to assure its safety; crisis situations should not bypass the need to establish a vaccine's risk-benefit profile and to address both real and perceived safety concerns (Lee *et al.* 2015, Rougeron *et al.* 2015).

To understand the possible use and limitations of vaccines in great apes (and humans), it is essential to understand that vaccine candidates differ in their composition; defining so-called different vaccine platforms (Marzi & Feldmann 2014). The composition of the vaccine determines which type and degree of immune reaction it stimulates, and if a vaccine needs to be injected rather than administered via other routes to induce sufficient protection from infection. Subsequently, this decides which type and how many interventions are necessary on the individual or population level to induce protection and how long this protection lasts.

Several Ebola vaccine candidates are vector-based vaccines that consist of some type of relatively benign virus that has been genetically modified to display Ebola specific antigens on the surface. In this way, the vector virus will present itself as Ebola virus to the vaccinated host and activate an appropriate immune response as long as it is viable. Such vaccine candidates include *cAd3-EBO-Z* and *rVSV-ZEBOV* which have both proven to be protective after one single injection in NHP-studies and are currently in human trials (Henao-Restrepo *et al.* 2015, De Santis *et al.* 2016). *rVSV-ZEBOV* can also be used when the individual is already infected with Ebola, and an important feature which might be relevant for the possible use in wild apes is that oral immunization with this vaccine has proven possible, at least in the mouse and macaque model, suggesting that baiting of vaccine is in theory possible (Qiu *et al.* 2009).

Another type of vector based vaccine candidate is Cytomegalovirus based vaccine (CMV vaccine) (Marzi *et al.* 2016). In contrast to the other candidates, this vaccine is intended specifically for wild great apes and is designed to be able to spread from one individual to another. Ideally the vaccine virus should be given to relatively few individuals and subsequently spread through the population indefinitely whilst stimulating protection against Ebola. However, the ethics and risks of introducing any genetically modified virus, even if the original vector virus is naturally found in the population, requires careful and thorough discussion (Tsuda *et al.* 2011, Murthy *et al.* 2013). Once released, the vaccine-virus can no longer be removed from the population.

A vaccine with less safety concerns is Virus Like Particles-vaccine; it does not include any virus component but rather consists of a genetically engineered protein product that mimics the structure of EV (Marzi & Feldmann 2014). Studies in NHP, including captive chimpanzees, show that multiple injections are required to reach full potency of the vaccine, hence there are doubts about the use of this vaccine in the wild (Warfield *et al.* 2014).

Additional candidates have also proven protective in NHP models and vaccine research is currently progressing rapidly hence further candidates and possibly additional administration routes are likely to appear in the future (Bukreyev & Collins 2010, Geisbert 2015).

POSSIBLE VACCINATION STRATEGIES

The different properties of the vaccines determine how they can be administered, how many individuals might be protected after a single intervention and how long protection lasts. The selection of vaccine and cost-effective vaccination strategy are therefore mainly determined by 1) the access to the apes, i.e. if they are habituated to human presence or not, and 2) the aim of vaccination at the time, i.e. to prevent introduction of Ebola from the natural source into the ape population or to stop the spread of infection within populations once an outbreak has started. Wild apes that have been habituated for behavioral research or tourism are observed on a regular basis and individual apes are usually identified which means that initial vaccination, monitoring and possible follow-up vaccination are in theory possible, whereas unhabituated apes would not be accessible for such treatment. The majority of apes in Africa are not habituated although for some relatively small populations such as the Virunga Mountain Gorillas, a relatively large proportion (ca.70%) is habituated (Robbins *et al*. 2011). It should, however, be noted that these apes, including sanctuary apes (Anonymous 2016b), are wild and endangered although they are habituated, and should therefore not be considered guinea pigs for experimental vaccinations. For unhabituated apes, their population size and location, and even existence, might not be known, hence access to these apes and monitoring of vaccination coverage is a real challenge.

For these reasons it is possible that different vaccines and strategies are suitable according to the specific population in question. Strategies will be discussed below in general terms without referring to current candidates as additional candidates will likely become available (Figure 2).

Prevention of Ebola spill-over into habituated populations

The more or less constant proximity of habituated apes makes darting a possible route of vaccine administration. Although it can be challenging and possibly dangerous to the apes and personnel, darting yields a high degree of certainty that the vaccine has been received. Ideally a vaccine that protects after a single administration should be used to avoid excessive darting that might also have a negative impact on years or decades of habituation efforts. For the same reason the vaccine (with or without required booster) should induce long lasting protection. Although the vaccine candidates that are presently in human trials are considered to induce long-term protection sufficient to last for the duration of a human outbreak, a protection longevity of some months or even a couple of years might not be optimal for long term prevention of EVD in wild apes (Stanley *et al.* 2014, Wong *et al.* 2014).

Baiting is in theory another option for vaccine delivery to habituated apes, provided the vaccine is designed to induce protection after oral administration. However, apes are selective with what they eat hence it might be difficult to ensure that all individuals receive the vaccine or on the other hand that a single or few dominant individuals consume vaccine doses higher than intended (Ryan & Walsh 2011). Furthermore, feeding wild great apes with food items that might be desirable but foreign to them, e.g. bananas, is not a generally accepted practice; however, the cost-benefit of such provisioning for vaccination purpose might have to be reconsidered.

As the number of habituated great apes is relatively small, it could be possible to vaccinate all or at least the majority of them (figure 2b), provided the costs are covered. If not, specific members that are important for reasons such as breeding potential and/or disease spreading capabilities within the social network must be selected according to the specific species and demographic structure for the population in question (Carne *et al.* 2013). It should, however, be kept in mind that as long as the ecology and transmission of Ebola from the natural reservoir is not determined, high-risk individuals within a group cannot be identified. If a safe, efficient and ethically accepted self-spreading vaccine should become available for use in great apes (as suggested by Marzi and collegues (2016)), such selection would not be necessary and relatively few individuals would require darting to induce high level of vaccination cover and longevity of protection in a population (figure 2d).

Control of EVD outbreaks in habituated populations

Trying to vaccinate by darting during a great ape Ebola outbreak is likely to be stressful and dangerous for human personnel and apes. Human safety must be addressed in planning of any intervention whether the ape population is habituated or not. Further, the rapid progression of disease and death leaves little time to vaccinate which makes it more essential to follow a logistically feasible vaccination priority list to prevent further spread of infection. A 'ring vaccination' (figure 2c) of in-contact individuals and individuals in surrounding unaffected populations could be attempted (Henao-Restrepo *et al.* 2015). This approach might, however, not be fast or efficient enough to stop the outbreak, and virus could also possibly be introduced directly from the reservoir on the other side of the vaccination-borders (Leroy *et al.* 2004). In an outbreak situation, it would be beneficial to use a vaccine that is effective also when an individual is already infected. Protection induced by a potential self-spreading type of vaccine would require that the vaccine vector would spread and induce protection faster than the Ebola virus itself would spread; such a vaccine would make more sense to use well before an outbreak situation but could perhaps be beneficial if used in parallel with other vaccinations as an extension of a ring vaccination strategy.

An important issue is that a great ape outbreak might be accompanied by human cases. If there is low availability of vaccines developed for human use, one should seriously consider the cost-benefit and ethics of using these valuable doses on wildlife.

Prevention of Ebola spill-over into non-habituated populations

The elusive nature of non-habituated apes poses a considerable obstacle to administering injectable vaccines and it is questionable if vaccinating randomly encountered individuals would be sufficient to protect populations across Africa, especially if the vaccination would require booster injections after some time. The cost-benefit must be modelled for the individual populations, including factors such as to what proportion of the population can be expected to be reached, and the perceived risk of viral spill-over in the area. The unpredictable emergence of Ebola from its reservoir makes such risks difficult to estimate. Although relatively specific areas in North Eastern Gabon, ROC and Cote d'Ivoire have proven to be ecologically suitable for transmission of Ebola into great ape population, the majority of the tropical belt of Africa is currently considered risk area for viral emergence (Pigott *et al.* 2014, Walsh & Haseeb 2015).

Baiting does not require close-range contact with individuals, but poses the same challenges as described for habituated apes and also requires efficient administration on a much larger geographical scale. A self-spreading vaccine that would require relatively few interventions and which could in theory provide widespread protection against Ebola may seem promising for remote, non-accessible populations but the safety and ethics of such an approach must be addressed.

Other ideas would be to develop efficient fog sprays or environmental vaccines that can be administered to larger areas of remote ape habitats, or when the Ebola ecology is better understood, to vaccinate species that the apes could contract Ebola infection from.

Control of EVD outbreaks in non-habituated populations

Firstly, outbreaks in un-monitored populations are likely to go unnoticed or to be first recognized when it is large enough to be detected by wildlife surveys, unless there is an efficient hunter surveillance network already in place. Secondly, for reasons discussed above, reaching and vaccinating enough unhabituated individuals in order to control a rapidly spreading outbreak is unlikely. Ring vaccination of unaffected habituated groups might be considered to prevent spread of infection to groups outside of the ring, or to protect particularly vulnerable groups within the ring.

SUMMARY OF FEASABILITY AND CHALLENGES OF EFFICIENT VACCINATION OF WILD APES

There are currently a number of challenges to be overcome before vaccination can be efficient from a conservation perspective. Nevertheless, strategies should be formulated and discussed based on up-to-date knowledge and the prospects of future advances in vaccine development, to prepare financially and logistically for possibly rapid changes in vaccine availability. This needs to be done for each specific population to accommodate for differences in species, population size, habituation status, accessibility and other factors relevant to the countries in question. Management plans can be discussed and adjusted according to changes in the development, availability and cost of vaccines, disease situation, knowledge about Ebola ecology, and opinion on wildlife vaccination. To this end, interdisciplinary meetings including representatives from the fields of primatology, virology, wildlife conservation, ecology, epidemiology, virology, immunology and public health are necessary to discuss and find well-considered solutions for this complex topic. These discussions should also include how to record details of any vaccination attempt and how to monitor the effect in a scientific way (Travis *et al.* 2008). The more data that are available, the better we can implement disease models and adjust future disease prevention strategies.

NEED FOR GREAT APE HEALTH MONITORING, RESEARCH AND DISEASE AWARENESS

Irrespective if future strategies for preventing Ebola will be implemented or not, monitoring of great ape health itself is important and should be included into conservation plans together with poaching control and habitat protection (Travis *et al.* 2006). Further, the fact that apes and humans alike are highly susceptible to Ebola make the great apes important sentinels for human disease, therefore monitoring mortality in these wildlife hosts, and collecting samples for pathogen identification, remains an important corner stone to predict and possibly prevent zoonotic transmission into humans (Calvignac-Spencer *et al.* 2012). It is the responsibility of any conservation and research project to take wildlife death seriously, to develop protocols in the event of a disease outbreak and report cases even if the project itself is not engaged in great ape health. This should go hand in hand with raising public awareness about the risk of hunting and scavenging apes, and also highlighting the importance of hunters and other villagers reporting dead apes, perhaps via the chiefs of the villages to the local health authorities. During the first bout of human outbreaks in Gabon in the nineties, lack of awareness of the role of great apes in Ebola outbreaks limited the epidemiological and laboratory investigation of these species. The need for wildlife monitoring was subsequently recognized and in the next series of outbreaks some years later a well prepared wildlife monitoring network was able to warn the health authorities about the risk of human cases and sampling of carcasses lead to the confirmation of infected apes and duikers as root of the human outbreaks (Rouquet *et al.* 2005).

Great ape monitoring should be done in a systematic and quantitative manner by qualified personnel, such as veterinarians that have been trained to perform wild animal necropsies under highly stringent safety conditions. Such monitoring has been shown to be a prerequisite for early detection and effective sampling of suspected cases (Leendertz *et al.* 2006, Travis *et al.* 2008); laboratory analysis is the only way to confirm Ebola infection. Antibody testing of non-invasively collected great ape samples can show which population have been exposed to the virus and surveys to estimate group density and population numbers may inform about population declines possibly caused by Ebola (Cameron *et al.* 2016, Campbell *et al.* 2008, Kouakou *et al.* 2009, Olson *et al.* 2012, Reed *et al.* 2014). Identification of the virus' true reservoir and infection pathways in nature remains important research (Leendertz 2016). This emphasizes the need for continuous funding and recruitment of people involved with disease monitoring, ranging from veterinarians to field primatologists, project managers, ecologists, and laboratory workers. We encourage the involvement of researchers and laboratories in great ape countries for increased sustainability of such work.

Although not all human EVD outbreaks that have occurred can be linked to deaths in great apes, it is clear that contact with bush meat from any species of great ape either via hunting, scavenging or butchering, is a major risk factor for exposure to Ebola virus (Feldmann & Geisbert 2011, Anonymous 2014c). This epidemiologically and laboratory-confirmed transmission pathway should be a reminder that hunting animals that may be infected with EV can increase the risk of human outbreaks. Continuous public education by health and conservation organizations in great ape countries remains an important and priority task for disease prevention; a good example is the hunter surveillance and public health programme in Northern Congo (Anonymous 2015e). Such outreach programmes that communicate, in a culturally sensitive way, the dangers of handling bushmeat enhance awareness of zoonotic infections from great apes and other wildlife. Although it would not affect the occurrence of EVD in apes, there may be indirect benefits to conservation. Possible risks of disease transmission between apes and humans must also be conveyed to tourists that are in relatively close contact with wild great apes in tourism-for-conservation- projects (Muehlenbein & Ancrenaz 2009, Macfie & Williamson 2010).

WHAT TO DO IF EBOLA IS SUSPECTED OR CONFIRMED IN APES?

This review is not meant as a manual for collection and analyses of data and samples; for this we refer to other guidelines for great ape health monitoring (Gilardi *et al.* 2015). First and most important is to never forget that the Ebola virus is highly infectious and highly lethal and represents a major risk to human health; well-meaning, unskilled or unprotected investigators or tourists as well as highly-trained public health workers might risk their own life and possibly initiate a human outbreak. The rapid decomposition of carcasses in the rainforest means that finding a single carcass is very rare, hence encountering even one dead ape should raise awareness as this individual might be one of many other undetected carcasses and an immediate and intense search should be initiated. Whenever an ape carcass is detected, especially if Ebola is suspected, the wildlife authorities and the local ministry of health should be informed, following the ROC surveillance guidelines (Reed *et al.* 2014). Necropsy samples should only be sampled by specialists trained for these situations, and anybody in direct or indirect contact with carcasses must adhere to the highest standard of protective clothing and disinfection (Leendertz *et al.* 2006, Ringen *et al.* 2015).

The possibility and limitations of vaccination in an outbreak situation, as described above, should ideally have been discussed well beforehand and a network of assistance for such emergency situations should be established. Finally, openly sharing experiences after an outbreak is crucial for improving preparedness for future outbreaks.

CONCLUSION

Ebola Viruses can cause large epidemics in humans and great apes. The likelihood that these viruses will continue to emerge unpredictably in tropical Africa highlights the necessity to protect apes from the severe impact of EVD and to reduce human contact to infected wildlife sources. Further research is required to determine the natural circulation of EVs and the mechanisms facilitating viral emergence; however, bushmeat from infected apes is confirmed as source of infection for human outbreaks hence public education of zoonotic diseases and monitoring of great ape health remain important human outbreak prevention strategies. With the rapid progress in Ebola vaccine development, vaccination of wild great apes might become a tool for conservation and protection of human health in the future. Research must focus on developing safe and efficient safe vaccines that can be delivered efficiently to larger populations of elusive wild apes in their natural remote habitats. A thorough understanding of the disease and the great apes' natural behavior as well as knowledge of the properties of vaccine candidates is necessary to assess the feasibility of potential vaccination programs. This article describes the occurrence of EVD in great apes and discusses the possibilities and limitations of vaccinating wild great apes against Ebola.

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FIGURE LEGENDS

Figure 1. Overview of Ebola-positive great ape carcasses and points of origin of human outbreaks across the tropical belt of Africa. The larger green areas in Gabon and ROC represent the three and five outbreaks, respectively (Pigott *et al.* 2014, Anonymous 2015c)

Figure 2. Schematic presentation of the principles of Ebola vaccination strategies in great apes

A- vaccination border for prevention of spillover from natural reservoir or other hosts; B- vaccination of specific groups, i.e. habituated groups; C-ring vaccination in the event of an EVD outbreak; D- the principle of a self-spreading vaccine. The bats represent any possible source of Ebola infection. The open dots and circles on the grey background illustrate individuals and groups in the ape population. Black dots are Ebola infected apes; red dots are vaccinated apes.

Red arrows indicate introduction of vector virus, the orange dots and arrows shows subsequent spread, white arrows indicate route of even further spread.

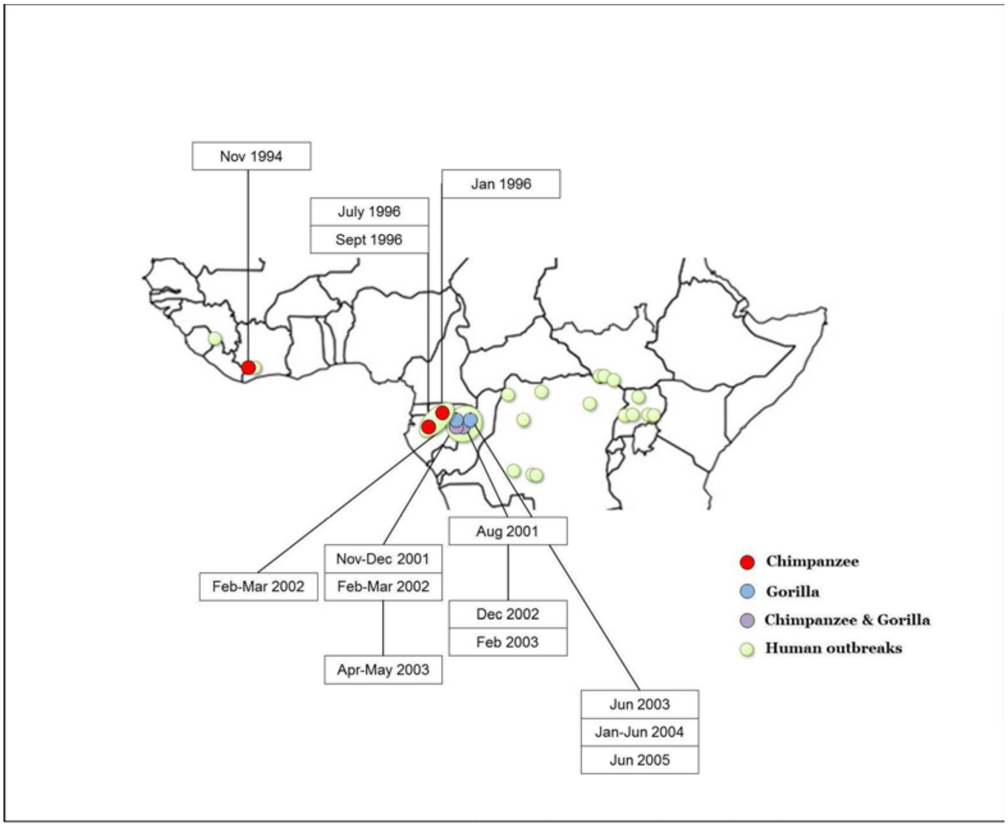

Fig. 1.

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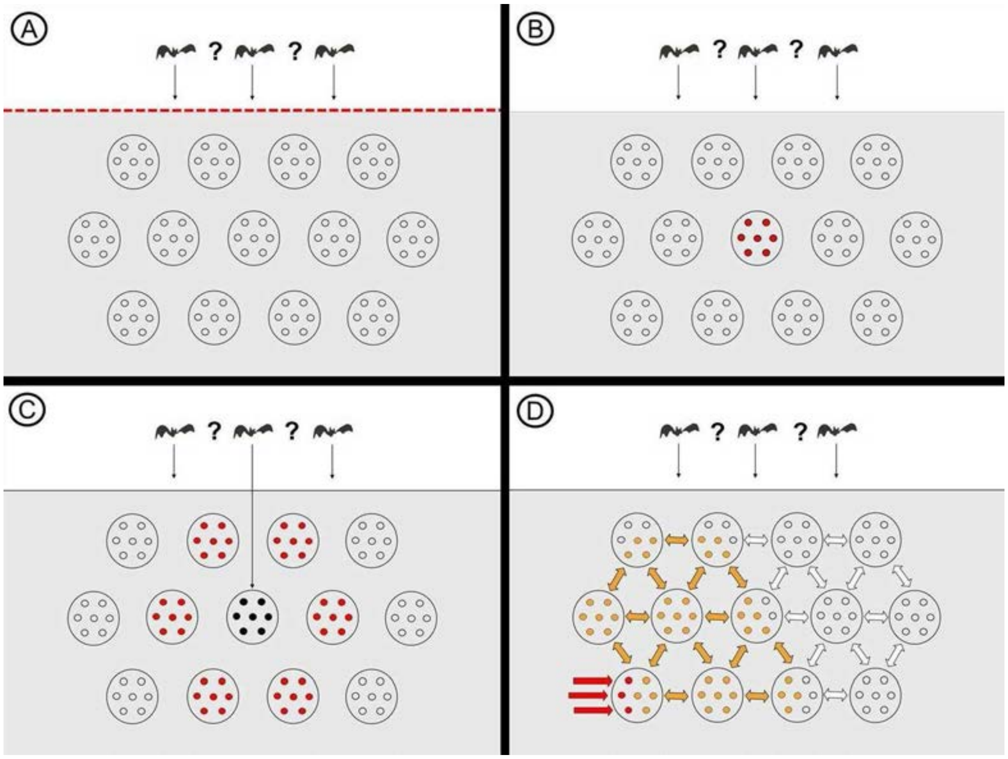

Fig. 2

Fig. 2

252x189mm (150 x 150 DPI)