



## Ebola in great apes – current knowledge, possibilities for vaccination and the implications for conservation and human health

Journal: *Mammal Review*

Manuscript ID: MAMMAL-16-22.R1

Manuscript Type: Review

Keywords: Ebola, wild great apes, vaccination, conservation, global health

Subject Areas (select one): Conservation/management

Mammalian Orders (select all that apply): Primates

SCHOLARONE™  
Manuscripts

Only

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

4  
5 **ABSTRACT**

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

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

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

22

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

24 **RUNNING HEAD:** Ebola in great apes

25 **WORD COUNT: 9945**

26

27 **INTRODUCTION**

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

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

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

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

62

### 63 **EBOLA VIRUS DISEASE**

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

73 The biological aspects of the EVD disease process are similar in humans and apes (Kuhn  
74 2008). EVs are highly infectious and enter the body through contact with mucus membranes,  
75 eyes, and broken skin. After an incubation period of 2-21 days, the infected individual

76 develops symptoms which include fever, vomiting, diarrhoea, internal and sometimes external  
77 bleeding. There are few observations of wild apes that have presumably been suffering from  
78 EVD, but signs of abdominal pain, lethargy, poor appetite, diarrhoea and emaciation or  
79 simply “abnormal behaviour” have been noted in great apes, and bleeding from the nostrils  
80 has been observed among other non-human primates (NHPs) (Formenty *et al.* 1999, Georges  
81 *et al.* 1999, Lahm *et al.* 2007). Other individuals can get infected through contact with the  
82 body or body fluids from a sick or dead member of the social group (Warren & Williamson  
83 2004). It is debated how infection can spread through ape populations over large geographical  
84 areas and there are two main theories. The first theory proposes that the spread occurs solely  
85 by group-to-group infection after a single or few introductions of the virus from the original  
86 reservoir or other infected animals into an uninfected ape population. Such spread could occur  
87 via direct contact such as intergroup encounters, extra-community copulation, and emigration  
88 of females (including infected gorilla females leaving the group after death of the silverback),  
89 or indirectly, via sharing of feeding sites or home range (Vigilant *et al.* 2001, Bermejo 2004,  
90 Bradley *et al.* 2004). EV has been isolated in semen of infected human males many months  
91 after infection (Chughtai *et al.* 2016) indicating that surviving male apes could initiate new  
92 infection chains if mating occurs within the infectious period. The potential for group-to-  
93 group spread in apes depends on several factors including the incubation time of the specific  
94 individual (or longevity of infectious semen), travel distance and ape density (Walsh *et al.*  
95 2007, Walsh *et al.* 2009, Ryan *et al.* 2013). The sociality of African apes, including the  
96 natural fission-fusion social system of chimpanzees (Lehmann & Boesch 2004) will also  
97 influence the spread of virus in a positive or negative way, depending on the contact pattern  
98 and which individuals are infected.

99 The second theory of how infection can spread over large areas proposes that outbreaks occur  
100 mainly as a direct consequence of repeated spill-over from a so far unidentified natural host  
101 into geographically separated ape social groups. The outbreak distribution is consequently

102 defined by the dispersal and movements of the reservoir host itself rather than the apes (Leroy  
103 *et al.* 2004). However, these two theories are not mutually exclusive, as it is possible that both  
104 these modes of transmission are important, and that one or the other can predominate at times  
105 or sites depending on the underlying factors that trigger or sustain the outbreaks. In any case,  
106 Ebola's tremendous ability to spread rapidly once it has emerged is illustrated by the recent  
107 human outbreak in West Africa, which started with one infected child and subsequently  
108 included nearly 30,000 people (Mari Saez *et al.* 2015, Anonymous 2015c).

109 Experimental infections show that deaths in NHPs occur within a similar time frame as in  
110 humans (ca.8-10 days after onset of symptoms for ZEBOV and ca.12-14 days for TFEBOV)  
111 (Geisbert *et al.* 2009). Although the mortality rate can be extremely high (up to 88% in  
112 humans and possibly higher in great apes infected with ZEBOV [Bermejo *et al.* 2006,  
113 Anonymous 2015c]), it is evident that recovery and survival after infection is possible.  
114 Antibodies to Ebola virus have been detected in blood from humans, wild-born chimpanzees  
115 and other primates and in the feces from wild-living gorillas, proving that some individuals  
116 exposed to the virus can mount an immune response and recover after infection (Leroy *et al.*  
117 2004, Lahm *et al.* 2007, Reed *et al.* 2014). Survival of individuals in great ape groups whose  
118 other members have died of Ebola also suggest that recovery is possible (Formenty *et al.*  
119 1999, Bermejo *et al.* 2006); however, without laboratory testing of samples, it is not possible  
120 to ascertain whether these individuals were in fact infected or managed to escape exposure.  
121 The infectious, acute and often lethal nature of Ebola shows, however, that great apes are  
122 unlikely to be able to indefinitely sustain an infection chain.

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

127

## 128 OCCURENCE AND IMPACT OF EVD IN GREAT APES

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

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

141 The ZEBOV species has had a much more significant impact on great apes. Outbreaks have  
142 occurred in North Eastern Gabon and ROC in two clusters: first in the mid-1990s and then in  
143 the first five years of this century. Surveillance of wild great apes is challenging and does not  
144 cover all wild populations hence outbreaks could also have occurred undetected at other  
145 times. Most of what is known about the outbreaks in apes in North Eastern Gabon in the mid-  
146 1990s emanated from investigations of three human outbreaks that occurred at that time  
147 (Georges *et al.* 1999). In November 1994, there were concurrent reports of wildlife deaths  
148 among the local gorilla and chimpanzee populations when the first human cases appeared in  
149 gold mining camps in the Minkebé forest area. About a year later, butchering a dead  
150 chimpanzee found ca. 40 km further south initiated a second human outbreak. The third  
151 human outbreak started yet another 160 km further south six months later with the death of  
152 two hunters, of which one was said to have killed several mangabeys in the time before he got  
153 sick. Dead chimpanzees and gorillas were encountered on several occasions during and after

154 these outbreak years, indicating that EVD was affecting the apes for a long time (Georges *et*  
155 *al.* 1999, Lahm *et al.* 2007). From these dead apes, a skin sample tested positive in Ebola  
156 immunohistology staining.

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

164 A surveillance network was subsequently set up in North Eastern Gabon and ROC due to the  
165 association between human EVD outbreaks, the regularity of human contact with apes and/or  
166 ape meat in these countries and the decline of great ape populations. This network, which also  
167 included monitoring and sampling of a range of wildlife carcasses, revealed that five human  
168 EVD outbreaks that occurred between October 2001 and May 2005 resulted from contact to  
169 infected carcasses, mainly gorillas and chimpanzees (Leroy *et al.* 2004, Rouquet *et al.* 2005,  
170 Nkoghe *et al.* 2011). Ebola positive ape carcasses were found in several forest areas including  
171 Lossi and Odzala National Parks, ROC, where population losses exceeding 90% were  
172 observed in habituated gorillas (Bermejo *et al.* 2006). In Lossi alone Ebola was estimated to  
173 have killed 5,000 individuals. Ebola is believed to have caused ape deaths in Odzala until at  
174 least 2007 (Caillaud *et al.* 2006, Cameron *et al.* 2016), however, since 2005, no further cases  
175 of EVD in African apes have been confirmed. A human EVD outbreak in Boende, DRC, in  
176 2014 might have been linked to contact with a primate carcass (species unidentified)  
177 (Maganga *et al.* 2014), however, no samples have been obtained for testing and the link  
178 remains speculative. The human 2013-2016 EVD epidemic in West Africa is not believed to  
179 be associated with deaths of great apes or other primates, but is rather thought to be the



180 consequence of a child's accidental contact with a large colony of insectivorous bats (Mari  
181 Saez *et al.* 2014). The area where the epidemic started harbours few primates, no case of dead  
182 apes has been reported and recent surveys indicate no decline in primate populations  
183 compared with surveys conducted prior to the outbreak.

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

194

#### 195 **WHERE AND HOW DO THE APES GET INFECTED WITH EBOLA?**

196 It is evident from the disease's rapid spread and high mortality in great apes that these  
197 primates are neither natural, asymptomatic carriers of the virus nor able to sustain the  
198 infection indefinitely, which are both important criteria for a reservoir host (Olival & Hayman  
199 2014). The apes are rather so-called 'accidental hosts' who act as a source of infection for  
200 human outbreaks when they themselves are infected from another host(s) sharing the same  
201 habitat. Numerous carcasses of other animals found in the Gabon and ROC forests between  
202 1994 and 2005 indicate that many species may be susceptible to EV, although the virus has  
203 only been isolated from gorillas, chimpanzees and a duiker (*Cephalophus* sp.) despite  
204 intensive testing of wildlife carcasses in outbreak areas for decades (Leroy *et al.* 2004, Lahm  
205 *et al.* 2007, Wittmann *et al.* 2007). Laboratory analyses of samples from certain fruit eating

206 and insect eating bats indicate that these bats were able to host, possibly spread and indeed  
207 survive infection; however, the virus itself has never been isolated from these hosts and more  
208 research is necessary to confirm that the virus is circulating primarily in any of these species  
209 or if yet another host represents the true reservoir (Olival & Hayman 2014, Leendertz *et al.*  
210 2015). Until the natural history and ecology of Ebola are more fully characterized, it is only  
211 possible to speculate about if and how apes (and humans) get infected from bats or other  
212 species (Viana *et al.* 2014). Apes are not known to hunt bats, but could potentially come in  
213 direct contact with fruit bats roosting in trees where apes feed, or with insectivorous bats  
214 roosting in tree holes where the apes search for honey, insects or water (Nishida 2010).  
215 Alternatively they could get infected via excretions from any kind of bat or other animal when  
216 ingesting contaminated food via feces, urine or saliva, although such excretion of EV in  
217 naturally infected bats is yet to be confirmed (Olival & Hayman 2014). Direct contact with  
218 other infected hosts is another possible infection route, including hunting of monkeys or other  
219 prey that might be infected and/or during inspections of infected carcasses (Boesch & Boesch-  
220 Achermann 2000, Karesh *et al.* 2012). In the chimpanzee outbreak in Côte d'Ivoire, red  
221 colobus monkey predation was considered a risk factor (Formenty *et al.* 1999), but was not  
222 confirmed as a transmission route.

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

231

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

234 Considering the severe reduction in population number and dissemination of groups that  
235 follows EVD outbreaks in great apes it is highly questionable if leaving this disease to be  
236 regulated by nature itself is a viable option. Demographic structure changes (for example  
237 increased frequency of emigration to start new breeding groups) of populations after severe  
238 declines can indeed occur to favour population growth (Genton *et al.* 2012, Genton *et al.*  
239 2014, Reed *et al.* 2014), however, in the case of repeated outbreaks and added pressure from  
240 other infectious diseases, bushmeat hunting and deforestation these recovery strategies might  
241 not be sufficient to prevent extinction (Leendertz *et al.* 2006, Morgan & Sanz 2007, Kondgen  
242 *et al.* 2008, N'Goran *et al.* 2012). Apes have a relatively long generation time (Anonymous  
243 2014a) which means that an outbreak of a disease with high mortality rate such as EVD  
244 requires a very long recovery time for affected populations; some calculated that it would take  
245 the affected gorilla population in Lossi more than 130 years to recover to initial population  
246 numbers (Ryan & Walsh 2011). In addition, fragmentation of populations due to mortality  
247 also has a negative impact on the genetic diversity of the populations, which could exacerbate  
248 extinction risk especially when populations are reduced in size. Therefore, it is clear that  
249 efficient strategies to reduce the impact on EVD in wild population will be beneficial for  
250 conservation. Further, prevention or reduction of infectious diseases in wildlife, including  
251 preservation of natural ecosystem integrity and biodiversity, will ultimately have a positive  
252 impact on human health (Rabinowitz *et al.* 2013). It should however be noted that not all  
253 human EVD originated from infected great apes, hence such strategies will not prevent human  
254 exposure to EVs from other wildlife sources (Anonymous 2015a).

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

270 In human EVD outbreaks, isolation of infected cases remains an important control strategy  
271 (Anonymous 2015d). Protection of the great apes cannot rely upon similar isolation strategies,  
272 although it might naturally occur to some degree as apes sometimes temporarily leave the rest  
273 of the social group when experiencing signs of disease (Boesch & Boesch-Achermann 2000).  
274 It is evident that some isolated groups of chimpanzees and gorillas continued to thrive in one  
275 instance only 20km from an outbreak area (Lahm *et al.* 2007), hence geographical separation  
276 could theoretically provide protection. However, attempting to isolate social groups by cutting  
277 corridors in the forest in order to stop spread of infected apes is unlikely to be feasible,  
278 desirable or efficient for various reasons. Firstly, clearing of larger geographical areas in  
279 dense and remote rainforest areas is laboursome and in conflict with forest preservation, and  
280 as apes are able to cross large open areas the effort might not even be worthwhile (Haurez *et*

281 *al.* 2013). Further, should separation be successful it might create stress for the apes due to  
282 man-made disturbance and change in their territory, and in longer term compromise the  
283 population genetic viability. And finally, the virus could be introduced to other parts of the  
284 forest by reservoir hosts not restricted by human-made barriers, which make attempts to  
285 geographically separate ape groups to avoid spread of infection redundant.

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

297 It is not the first time that vaccines have been used in wild apes (Ryan & Walsh 2011). For  
298 example, small scale vaccination has been done in habituated (or reintroduced) populations  
299 for diseases like measles, poliomyelitis, tetanus, and anthrax (Goodall 1986, Hastings *et al.*  
300 1991, Ryan & Walsh 2011). Vaccinations were done through distance darting, injections  
301 when other interventions were undertaken under general anaesthesia, or in the case of  
302 chimpanzee poliomyelitis in the mid-1960s, through feeding of bananas; an acceptable  
303 practice at the time.

304

## 305 **THE PROSPECT OF VACCINATING WILD GREAT APES AGAINST EBOLA**

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

313

## 314 **CURRENT VACCINE CANDIDATES**

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

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

328 Several Ebola vaccine candidates are vector-based vaccines that consist of some type of  
329 relatively benign virus that has been genetically modified to display Ebola specific antigens

330 on the surface. In this way, the vector virus will present itself as Ebola virus to the vaccinated  
331 host and activate an appropriate immune response as long as it is viable. Such vaccine  
332 candidates include *cAd3-EBO-Z* and *rVSV-ZEBOV* which have both proven to be protective  
333 after one single injection in NHP-studies and are currently in human trials (Henao-Restrepo *et*  
334 *al.* 2015, De Santis *et al.* 2016). *rVSV-ZEBOV* can also be used when the individual is already  
335 infected with Ebola, and an important feature which might be relevant for the possible use in  
336 wild apes is that oral immunization with this vaccine has proven possible, at least in the  
337 mouse and macaque model, suggesting that baiting of vaccine is in theory possible (Qiu *et al.*  
338 2009).

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

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

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

356

357 **POSSIBLE VACCINATION STRATEGIES**

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

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

379

380 **Prevention of Ebola spill-over into habituated populations**



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

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

399 As the number of habituated great apes is relatively small, it could be possible to vaccinate all  
400 or at least the majority of them (figure 2b), provided the costs are covered. If not, specific  
401 members that are important for reasons such as breeding potential and/or disease spreading  
402 capabilities within the social network must be selected according to the specific species and  
403 demographic structure for the population in question (Carne *et al.* 2013). It should, however,  
404 be kept in mind that as long as the ecology and transmission of Ebola from the natural  
405 reservoir is not determined, high-risk individuals within a group cannot be identified. If a safe,  
406 efficient and ethically accepted self-spreading vaccine should become available for use in

407 great apes (as suggested by Marzi and colleagues (2016)), such selection would not be  
408 necessary and relatively few individuals would require darting to induce high level of  
409 vaccination cover and longevity of protection in a population (figure 2d).

410

#### 411 **Control of EVD outbreaks in habituated populations**

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

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

430

#### 431 **Prevention of Ebola spill-over into non-habituated populations**

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

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

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

452

### 453 **Control of EVD outbreaks in non-habituated populations**

454 Firstly, outbreaks in un-monitored populations are likely to go unnoticed or to be first  
455 recognized when it is large enough to be detected by wildlife surveys, unless there is an  
456 efficient hunter surveillance network already in place. Secondly, for reasons discussed above,  
457 reaching and vaccinating enough unhabituated individuals in order to control a rapidly

458 spreading outbreak is unlikely. Ring vaccination of unaffected habituated groups might be  
459 considered to prevent spread of infection to groups outside of the ring, or to protect  
460 particularly vulnerable groups within the ring.

461

## 462 **SUMMARY OF FEASIBILITY AND CHALLENGES OF EFFICIENT** 463 **VACCINATION OF WILD APES**

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

479

## 480 **NEED FOR GREAT APE HEALTH MONITORING, RESEARCH AND DISEASE** 481 **AWARENESS**

482 Irrespective if future strategies for preventing Ebola will be implemented or not, monitoring  
483 of great ape health itself is important and should be included into conservation plans together

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

501 Great ape monitoring should be done in a systematic and quantitative manner by qualified  
502 personnel, such as veterinarians that have been trained to perform wild animal necropsies  
503 under highly stringent safety conditions. Such monitoring has been shown to be a prerequisite  
504 for early detection and effective sampling of suspected cases (Leendertz *et al.* 2006, Travis *et*  
505 *al.* 2008); laboratory analysis is the only way to confirm Ebola infection. Antibody testing of  
506 non-invasively collected great ape samples can show which population have been exposed to  
507 the virus and surveys to estimate group density and population numbers may inform about  
508 population declines possibly caused by Ebola (Cameron *et al.* 2016, Campbell *et al.* 2008,  
509 Kouakou *et al.* 2009, Olson *et al.* 2012, Reed *et al.* 2014). Identification of the virus' true

510 reservoir and infection pathways in nature remains important research (Leendertz 2016). This  
511 emphasizes the need for continuous funding and recruitment of people involved with disease  
512 monitoring, ranging from veterinarians to field primatologists, project managers, ecologists,  
513 and laboratory workers. We encourage the involvement of researchers and laboratories in  
514 great ape countries for increased sustainability of such work.

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

530

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

532 This review is not meant as a manual for collection and analyses of data and samples; for this  
533 we refer to other guidelines for great ape health monitoring (Gilardi *et al.* 2015). First and  
534 most important is to never forget that the Ebola virus is highly infectious and highly lethal and  
535 represents a major risk to human health; well-meaning, unskilled or unprotected investigators

536 or tourists as well as highly-trained public health workers might risk their own life and  
537 possibly initiate a human outbreak. The rapid decomposition of carcasses in the rainforest  
538 means that finding a single carcass is very rare, hence encountering even one dead ape should  
539 raise awareness as this individual might be one of many other undetected carcasses and an  
540 immediate and intense search should be initiated. Whenever an ape carcass is detected,  
541 especially if Ebola is suspected, the wildlife authorities and the local ministry of health should  
542 be informed, following the ROC surveillance guidelines (Reed *et al.* 2014). Necropsy samples  
543 should only be sampled by specialists trained for these situations, and anybody in direct or  
544 indirect contact with carcasses must adhere to the highest standard of protective clothing and  
545 disinfection (Leendertz *et al.* 2006, Ringen *et al.* 2015).

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

550

## 551 **CONCLUSION**

552 Ebola Viruses can cause large epidemics in humans and great apes. The likelihood that these  
553 viruses will continue to emerge unpredictably in tropical Africa highlights the necessity to  
554 protect apes from the severe impact of EVD and to reduce human contact to infected wildlife  
555 sources. Further research is required to determine the natural circulation of EVs and the  
556 mechanisms facilitating viral emergence; however, bushmeat from infected apes is confirmed  
557 as source of infection for human outbreaks hence public education of zoonotic diseases and  
558 monitoring of great ape health remain important human outbreak prevention strategies. With  
559 the rapid progress in Ebola vaccine development, vaccination of wild great apes might  
560 become a tool for conservation and protection of human health in the future. Research must  
561 focus on developing safe and efficient safe vaccines that can be delivered efficiently to larger

562 populations of elusive wild apes in their natural remote habitats. A thorough understanding of  
563 the disease and the great apes' natural behavior as well as knowledge of the properties of  
564 vaccine candidates is necessary to assess the feasibility of potential vaccination programs.  
565 This article describes the occurrence of EVD in great apes and discusses the possibilities and  
566 limitations of vaccinating wild great apes against Ebola.

567

#### 568 **ACKNOWLEDGEMENTS**

569 This study was commissioned and funded by the United Nations Environmental Programme  
570 Great Apes Survival Partnership (GRASP). We thank Charles Emil for the assistance with the  
571 figures. Special thanks to Laura Darby and Nicolien de Lange for their assistance in editing  
572 the manuscript.

573

#### 574 **REFERENCES**

575 Anonymous (2014a) *State of the Apes: Extractive Industries and Ape Conservation*.  
576 Cambridge University Press, Cambridge, United Kingdom.

577

578 Anonymous (2014b) *Regional Action Plan for the Conservation of Western Lowland Gorillas*  
579 *and Central Chimpanzees 2015–2025*. IUCN, Gland, Switzerland.

580

581 Anonymous (2014c) An update on the risk of transmission of Ebola virus (EBOV) via the  
582 food chain. *European Food Safety Authority Journal* 12, 25.

583

584 Anonymous (2015a) Centre for Disease Control and Prevention. Outbreaks chronology: Ebola  
585 Virus Disease. <http://www.cdc.gov/vhf/ebola/outbreaks/history/chronology.html>.

586



587 Anonymous (2015b) World Health Organisation Ebola virus diseasefactsheet.

588 <http://www.who.int/mediacentre/factsheets/fs103/en/>.

589

590 Anonymous (2015c) World Health Organisation Ebola Situation Reports.

591 <http://apps.who.int/ebola/en/ebola-situation-reports>.

592

593

594 Anonymous (2015d) World Health Organisation. Manual for the care and management of  
595 patients in Ebola Care Units/ Community Care Centres.

596 [http://apps.who.int/iris/bitstream/10665/149781/1/WHO\\_EVD\\_Manual\\_ECU\\_15.1\\_eng.pdf?](http://apps.who.int/iris/bitstream/10665/149781/1/WHO_EVD_Manual_ECU_15.1_eng.pdf?ua=1)  
597 [ua=1](http://apps.who.int/iris/bitstream/10665/149781/1/WHO_EVD_Manual_ECU_15.1_eng.pdf?ua=1).

598

599 Anonymous (2015e) Wildlife Conservation Society. [http://www.wcs.org/conservation-](http://www.wcs.org/conservation-challenges/wildlife-health/disease-investigations/ebola.aspx)  
600 [challenges/wildlife-health/disease-investigations/ebola.aspx](http://www.wcs.org/conservation-challenges/wildlife-health/disease-investigations/ebola.aspx).

601

602 Anonymous (2016a) The IUCN Red List of Threatened Species. <http://www.iucnredlist.org/>.

603

604 Anonymous (2016b) Pan African Sanctuaries Alliance <http://www.pasaprimates.org>

605

606 Bermejo M (2004) Home-range use and intergroup encounters in western gorillas (*Gorilla g.*  
607 *gorilla*) at Lossi forest, North Congo. *American Journal of Primatology* 64:223-232.

608

609 Bermejo M, Rodriguez-Teijeiro J D, Illera G, Barroso A, Vila C, Walsh PD (2006) Ebola  
610 outbreak killed 5000 gorillas. *Science* 314:1564.

611

- 612 Boesch C, Boesch-Achermann H (2000) *The Chimpanzees of the Tai Forest: Behavioural*  
613 *Ecology and Evolution*. Oxford University Press, Oxford, United Kingdom.
- 614
- 615 Bradley BJ, Doran-Sheehy D M, Lukas D, Boesch C, Vigilant L (2004) Dispersed male  
616 networks in western gorillas. *Current Biology* 14:510-513.
- 617
- 618 Bukreyev A, Collins PL (2010) Filovirus vaccines: what challenges are left? *Expert Review*  
619 *of Vaccines* 9:5-8.
- 620
- 621 Caillaud D, Levrero F, Cristescu R, Gatti S, Dewas M, Douadi M et al. (2006) Gorilla  
622 susceptibility to Ebola virus: the cost of sociality. *Current Biology* 16:489-491.
- 623
- 624 Calvignac-Spencer S, Leendertz SA, GillespieTR, Leendertz FH (2012) Wild great apes as  
625 sentinels and sources of infectious disease. *Clinical Microbiology and Infection* 18: 521-527.
- 626
- 627 Cameron KN, Reed P, Morgan DB, Ondzie AI, Sanz CM, Kühl HS, Olson SH, Leroy E,  
628 Karesh WB, Mundry R (2016) Spatial and Temporal Dynamics of mortality event among  
629 Central African Great Apes. *PLOS One* 11(5): e0154505
- 630
- 631 Campbell G, Kuehl H, N'Goran K , Boesch C (2008) Alarming decline of West African  
632 chimpanzees in Cote d'Ivoire. *Current Biology* 18:903-904.
- 633
- 634 Carne C, Semple S, Morrogh-Bernard H, Zuberbühler K, Lehmann J (2013) Predicting the  
635 Vulnerability of Great Apes to Disease: The Role of Superspreaders and Their Potential  
636 Vaccination. *PLOS One* 8:e84642
- 637

638 Chughtai AA, Barnes M, Macintyre CR (2016) Persistence of Ebola virus in various body  
639 fluids during convalescence: evidence and implications for disease transmission and control.  
640 *Epidemiology and Infection* 25:1-9.

641

642 Cohen J, Kupferschmidt K (2014) Infectious Diseases. Ebola vaccine trials raise ethical  
643 issues. *Science* 346:289-290.

644

645 De Santis O, Audran R, Pothin E, Warpelin-Decrausaz L, Vallotton L, Wuerzner G *et al.*  
646 (2016) Safety and immunogenicity of a chimpanzee adenovirus-vectored Ebola vaccine in  
647 healthy adults: a randomised, double-blind, placebo-controlled, dose-finding, phase 1/2a  
648 study. *Lancet Infectious Disease* 16:311-320.

649

650 Falzarano D (2016) Ebola vaccines: we have options. *Lancet Infectious Disease* 16:267-268.

651

652 Fedigan LM (2010) Ethical issues faced by field primatologists: asking the relevant questions.  
653 *American Journal of Primatology* 72:754-771.

654

655 Feldmann H, Geisbert TW (2011) Ebola haemorrhagic fever. *The Lancet* 377:849-862.

656

657 Formenty P, Boesch C, Wyers M, Steiner C, Donati F, Dind F *et al.* (1999) Ebola virus  
658 outbreak among wild chimpanzees living in a rain forest of Cote d'Ivoire. *Journal of*  
659 *Infectious Diseases* 179:120-126.

660

661 Geisbert TW (2015) Emergency treatment for exposure to Ebola virus: the need to fast-track  
662 promising vaccines. *Journal of the American Medical Association* 313:1221-1222.

663

- 664 Geisbert TW, Geisbert J B, Leung A, Daddario-DiCaprio KM, Hensley LE, Grolla A et al.  
665 (2009) Single-injection vaccine protects nonhuman primates against infection with marburg  
666 virus and three species of ebola virus. *Journal of Virology* 83:7296-7304.
- 667
- 668 Genton C, Cristescu R, Gatti S, Levrero F, Bigot E, Caillaud D et al. (2012) Recovery  
669 potential of a western lowland gorilla population following a major Ebola outbreak: results  
670 from a ten year study. *PLoS One* 5: e37106.
- 671
- 672 Genton C, Pierre A, Cristescu R, Levrero F, Gatti S, Pierre JS et al. (2014) How Ebola  
673 impacts social dynamics in gorillas: a multistate modelling approach. *Journal of Animal*  
674 *Ecology* 84:166-76.
- 675
- 676 Georges AJ, Leroy E M, Renaut AA, Benissan CT, Nabias RJ, Ngoc MT *et al.* (1999) Ebola  
677 hemorrhagic fever outbreaks in Gabon, 1994-1997: epidemiologic and health control issues.  
678 *Journal of Infectious Disease* 179:65-75.
- 679
- 680 Gilardi KV, Gillespie TR, Leendertz, FH, Macfie EJ, Travis DA, Whittier CA, Williamson  
681 EA (2015) *Best Practice Guidelines for Health Monitoring and Disease Control in Great Ape*  
682 *Populations*. ICUN SSC Primate Specialist Group, Gland, Switzerland.
- 683
- 684 Goodall J (1986) *The chimpanzees of Gombe: Patterns of behaviour*. Harvard University  
685 Press, Cambridge, United Kingdom.
- 686
- 687 Gruen L, Fultz A, Pruett J (2013) Ethical issues in African great ape field studies. *Institute for*  
688 *Laboratory Animal Research Journal* 54:24-32.

689

690 Hastings BE, Kenny D, Lowenstine LJ, and Foster JW (1991) Mountain gorillas and measles:  
691 ontogeny of a wildlife vaccination program. *Proceedings of American Association of Zoo*  
692 *Veterinarians* 198-205.

693

694 Haurez B, Petre CA, Doucet JL (2013) Impacts of logging and hunting on western lowland  
695 gorilla (*Gorilla gorilla gorilla*) populations and consequences for forest regeneration. A  
696 review. *Biotechnologie, Agronomie, Société et Environnement* 17:364-372.

697

698 Henao-Restrepo AM, Longini IM, Egger M, Dean NE, Edmunds WJ, Camacho A et al.  
699 (2015) Efficacy and effectiveness of an rVSV-vectored vaccine expressing Ebola surface  
700 glycoprotein: interim results from the Guinea ring vaccination cluster-randomised trial.  
701 *Lancet* 386:857-866.

702

703 Hockings KJ, McLennan MR, Carvalho S, AncrenazM, Bobe R, Byrne RW et al. (2015) Apes  
704 in the Anthropocene: flexibility and survival. *Trends in Ecology and Evolution* 30:215-222.

705

706 Hopkin M (2007) Gorillas on the list. *Nature* 449:127.

707

708 Huijbregts B, Wachter PD, Ndong Obiang LS, Akou ME (2003) Ebola and the decline of  
709 gorilla (*Gorilla gorilla*) and chimpanzee (*Pan troglodytes*) populations in Minkebe Forest,  
710 north-eastern Gabon. *Oryx* 37:437-443.

711

712 Karesh W, Reed P (2005) Ebola and great apes in Central Africa: current status and future  
713 needs. *Bulletin de la Société de pathologie exotique* 98:237-238.

714

- 715 Karesh WB, Dobson A, Lloyd-Smith JO, Lubroth J, Dixon MA, Bennett M et al. (2012)  
716 Ecology of zoonoses: natural and unnatural histories. *Lancet* 380:1936-1945.  
717
- 718 Kondgen S, Kuhl H, N'Goran PK, Walsh PD, Schenk S, Ernst N et al. (2008) Pandemic  
719 human viruses cause decline of endangered great apes. *Current Biology* 18:260-264.  
720
- 721 Kouakou CY, Boesch C, Kuehl H (2009) Estimating chimpanzee population size with nest  
722 counts: validating methods in Tai National Park. *American Journal of Primatology* 71:447-  
723 457.  
724
- 725 Kuhn JH (2008) Filoviruses. A compendium of 40 years of epidemiological, clinical, and  
726 laboratory studies. *Archives of Virology Supplement* 20:13-360.  
727
- 728 Kuhn JH, Becker S, Ebihara H, Geisbert TW, Johnson KM, Kawaoka Y et al. (2010)  
729 Proposal for a revised taxonomy of the family Filoviridae: classification, names of taxa and  
730 viruses, and virus abbreviations. *Archives of Virology* 155:2083-2103.  
731
- 732 Lahm SA, Kombila M, Swanepoel R, Barnes RF (2007) Morbidity and mortality of wild  
733 animals in relation to outbreaks of Ebola haemorrhagic fever in Gabon, 1994-2003.  
734 *Transactions of the Royal Society of Tropical Medicine and Hygiene* 101:64-78.  
735
- 736 Laurance WF, Goosem M, Laurance SGW (2009) Impacts of roads and linear clearings on  
737 tropical forests. *Trends in Ecology and Evolution* 24:659-669.  
738
- 739 Lee BY, Moss WJ, Privor-Dumm L, Constenla DO, Knoll MD, O'Brien KL (2015) Is the  
740 world ready for an Ebola vaccine? *Lancet* 385:203-204.

741

742 Leendertz FH, Pauli G, Maetz-Rensing K, Boardman W, Nunn C, Ellerbrok H, Jensen SA,  
743 Junglen S, Boesch C (2006) Pathogens as drivers of population declines: The importance of  
744 systematic monitoring in great apes and other threatened mammals. *Biological Conservation*  
745 131:325-337.

746

747 Leendertz SAJ (2016) Testing new hypotheses regarding Ebolavirus reservoirs. *Viruses* 8(2)

748

749 Leendertz SA, Gogarten JF, D ux A, Calvignac-Spencer S, Leendertz F (2016) Assessing the  
750 evidence of fruit bats as the reservoir for Ebola viruses. *Ecohealth* 13:18-25.

751

752 Lehmann J, Boesch C (2004) To fission or to fusion: effects of community size on wild  
753 chimpanzee (*Pan troglodytes verus*) social organisation. *Behavioural Ecology & Sociobiology*  
754 56:207-216

755

756 Leroy EM, Rouquet P, Formenty P, Souquiere, S, Kilbourne A, Froment JM et al. (2004)  
757 Multiple Ebola virus transmission events and rapid decline of central African wildlife. *Science*  
758 303:387-390.

759

760 Leroy EM, Telfer P, Kumulungui B, Yaba P, Rouquet P, Roques P et al. (2004) A serological  
761 survey of Ebola virus infection in central African nonhuman primates. *Journal of Infectious*  
762 *Diseases* 190:1895-1899.

763

764 Macfie EJ, Williamson EA (2010) *Best practice guidelines for great ape tourism*. IUCN/SSC  
765 Primate Specialist Group, Gland, Switzerland.

766

767 Maganga GD, Kapetshi J, Berthet N, Ilunga BK, Kingebeni PM et al. (2014) Ebola Virus  
768 Disease in the Democratic Republic of Congo. *New England Journal of Medicine* 371:2083-  
769 91

770

771 Maisels F, Ella Akou M, Douckaga M, Moundounga A (2004) *Mwagne National Park,*  
772 *Gabon: large mammals & human impact.* WCS/ WWF, Gabon.

773

774 Mari Saez A, Weiss S, Nowak K, Lapeyre V, Zimmermann F, Dux A et al. (2014)  
775 Investigating the zoonotic origin of the West African Ebola epidemic. *EMBO Molecular*  
776 *Medicine* 7:17-23.

777

778 Marzi A, Feldmann H (2014) Ebola virus vaccines: an overview of current approaches.  
779 *Expert Review of Vaccines* 13:521-531.

780

781 Marzi A, Murphy AA, Feldmann H, Parkins CJ, Haddock E, Hanley PW et al. (2016)  
782 Cytomegalovirus-based vaccine expressing Ebola virus glycoprotein protects nonhuman  
783 primates from Ebola virus infection. *Scientific Reports* 6:21674.

784

785 Morgan D, Sanz C (2007) Best practice guidelines for reducing the impact of commercial  
786 logging on great apes in Western Equatorial Africa. ICUN SSC Primate Specialist Group,  
787 Gland, Switzerland.

788



789 Muehlenbein MP, Ancrenaz M (2009) Minimizing pathogen transmission at primate  
790 ecotourism destinations : the need for input from travel medicine. *Journal of Travel*  
791 *Medicine* 16:229-232.

792

793 Murthy S, Couacy-Hymann E, Metzger S, Nowak K, De Nys H, Boesch C et al. (2013)  
794 Absence of frequent herpesvirus transmission in a nonhuman primate predator-prey system in  
795 the wild. *Journal of Virology* 87:10651-10659.

796

797 N'Goran PK, Boesch C, Mundry R, N'Goran EK, Herbinger I, Yapi F A et al. (2012) Hunting,  
798 law enforcement, and African primate conservation. *Conservation Biology* 26:565-571.

799

800 Nishida T, Zamma K, Matsusaka T, Inaba A (2010) *Chimpanzee Behavior in the Wild*.  
801 Springer, Tokyo, Japan.

802

803 Nkoghe D, Kone ML, Yada A, Leroy E (2011) A limited outbreak of Ebola haemorrhagic  
804 fever in Etoumbi, Republic of Congo, 2005. *Transactions of the Royal Society of Tropical*  
805 *Medicine and Hygiene* 105:466-472.

806

807 Olival KJ, Hayman DT (2014) Filoviruses in bats: current knowledge and future directions.  
808 *Viruses* 6:1759-1788.

809

810 Olson SH, Reed P, Cameron KN, Ssebide BJ, Johnson CK, Morse SS et al. (2012) Dead or  
811 alive: animal sampling during Ebola hemorrhagic fever outbreaks in humans. *Emerging*  
812 *Health Threats Journal*

813 5.

814

815 Olson SH, Reed P, Ondzie A, Cameron K, Joly D (2012) Maximizing nonhuman primate  
816 fecal sampling in the Republic of Congo. *Journal Of Wildlife Diseases* 48:888-898  
817

818 Pigott DM, Golding N, Mylne A, Huang Z, Henry AJ, Weiss DJ et al.(2014) Mapping the  
819 zoonotic niche of Ebola virus disease in Africa. *Elife*, 3.  
820

821 Qiu X, Fernando L, Alimonti JB, Melito PL, Feldmann F, Dick D et al. (2009) Mucosal  
822 immunization of cynomolgus macaques with the VSVDeltaG/ZEBOVGP vaccine stimulates  
823 strong ebola GP-specific immune responses. *PLoS One*, 4:e5547.  
824

825 Rabinowitz PM, Kock R, Kachani M, Kunkel R, Thomas J, Gilbert J et al. (2013) Toward  
826 proof of concept of a one health approach to disease prediction and control. *Emerging*  
827 *Infectious Diseases*19:doi:10.3201  
828

829 Reed PE, Mulangu S, Cameron KN, Ondzie AU, Joly D, Bermejo M et al. (2014) A new  
830 approach for monitoring ebolavirus in wild great apes. *PLoS Neglected Tropical Diseases* 8:  
831 e3143.  
832

833 Ringen K, Landrigan PJ, Duffy R, Melius J, McDiarmid MA (2015) Occupational safety and  
834 health protections against Ebola virus disease. *American Journal of Industrial Medicine*  
835 58:703-14  
836

837 Robbins MM, Gray M, Fawcett KA, Nutter FB, Uwingeli P, Mburanumwe I et al. (2011)  
838 Extreme conservation leads to recovery of the Virunga mountain gorillas. *PLoS One*  
839 6:e19788.  
840

- 841 Rougeron V, Feldmann H, Grard G, Becker S, Leroy EM (2015) Ebola and Marburg  
842 haemorrhagic fever. *Journal of Clinical Virology* 64:111-119.
- 843
- 844 Rouquet P, Froment JM, Bermejo M, Kilbourn A, Karesh W, Reed P et al. (2005) Wild  
845 animal mortality monitoring and human Ebola outbreaks, Gabon and Republic of Congo,  
846 2001-2003. *Emerging Infectious Diseases* 11:283-290.
- 847
- 848 Ryan SJ, Jones JH, Dobson AP (2013) Interactions between social structure, demography, and  
849 transmission determine disease persistence in primates. *PLoS One* 8:e76863.
- 850
- 851 Ryan SJ, Walsh, PD (2011) Consequences of non-intervention for infectious disease in  
852 African great apes. *PLoS One* 6:e29030.
- 853
- 854 Stanley DA, Honko AN, Asiedu C, Trefry JC, Lau-Kilby AW, Johnson JC et al. (2014)  
855 Chimpanzee adenovirus vaccine generates acute and durable protective immunity against  
856 ebolavirus challenge. *Nature Medicine* 20:1126-9
- 857
- 858 Towner JS, Sealy TK, Khristova ML, Albarino CG, Conlan S, Reeder SA et al. (2008) Newly  
859 discovered ebola virus associated with hemorrhagic fever outbreak in Uganda. *PLoS*  
860 *Pathogen* 4:e1000212.
- 861
- 862 Travis DA, Hungerford L, Engel GA, Jones-Engel L (2006) Disease risk analysis: a tool for  
863 primate conservation planning and decision making. *American Journal of Primatology*  
864 68:855-867.
- 865

- 866 Travis DA, Lonsdorf EV, Mlengeya T, Raphael J (2008) A science-based approach to  
867 managing disease risks for ape conservation. *American Journal of Primatology* 70:745-750.  
868
- 869 Tsuda Y, Caposio P, Parkins CJ, Botto S, Messaoudi I, Cicin-Sain L, et al. (2011) A  
870 replicating cytomegalovirus-based vaccine encoding a single Ebola virus nucleoprotein CTL  
871 epitope confers protection against Ebola virus. *PLoS Neglected Tropical Diseases* 5:e1275.  
872
- 873 Viana M, Mancy R, Biek R, Cleaveland S, Cross PC, Lloyd-Smith JO et al. (2014)  
874 Assembling evidence for identifying reservoirs of infection. *Trends in Ecology and Evolution*  
875 29:270-279.  
876
- 877 Vigilant L, Hofreiter M, Siedel H, Boesch C (2001) Paternity and relatedness in wild  
878 chimpanzee communities. *Proceedings of the National Acadademy of Sciences of the United*  
879 *States of America*, 98:12890-12895.  
880
- 881 Vogel G (2006) Ecology. Tracking Ebola's deadly march among wild apes. *Science* 314:1522-  
882 1523.  
883
- 884 Waldman L (2015) Urbanisation, the Peri-urban growth and Zoonotic disease. *Institute of*  
885 *developmental Studies Practice Paper in Brief* 22.  
886
- 887 Walsh PD, Tutin CEG, Oates, JF, Baillie JEM, Maisels F, Stokes EJ et al. (2007) IUCN Red  
888 List of Threatened Species. <http://www.iucnredlist.org/details/9404/0>  
889
- 890 Walsh PD, Bermejo M, Rodriguez-Teijeiro JD (2009) Disease avoidance and the evolution of  
891 primate social connectivity: Ebola, bats, gorillas, and chimpanzees. In Huffman MA &

892 Chapman CA (eds) *Primate Parasite Ecology: The Dynamics and Study of Host-Parasite*  
893 *Relationships*, 183-198. Cambridge University Press, Cambridge, United Kingdom.

894

895 Walsh PD, Breuer T, Sanz C, Morgan D, Doran-Sheehy D (2007) Potential for Ebola  
896 transmission between gorilla and chimpanzee social groups. *The American Naturalist*  
897 169:684-689.

898

899 Walsh MG, Haseeb MA (2015) The landscape configuration of zoonotic transmission of  
900 Ebola virus disease in West and Central Africa: interaction between population density and  
901 vegetation cover *Peer J* 3:e735

902

903 Warfield KL, Goetzmann, JE, Biggins JE, Kasda MB, Unfer RC, Vu H et al. (2014)  
904 Vaccinating captive chimpanzees to save wild chimpanzees. *Proceedings of the National*  
905 *Acadademy of Sciences of the United States of America* 111:8873-8876.

906

907 Warren Y, Williamson EA (2004) Transport of dead infant mountain gorillas by mothers and  
908 unrelated females. *Zoo Biology* 23:375-378.

909

910 Wittmann TJ, Biek R, Hassanin A, Rouquet P, Reed P, Yaba P et al. (2007) Isolates of Zaire  
911 ebolavirus from wild apes reveal genetic lineage and recombinants. *Proceedings of the*  
912 *National Acadademy of Sciences of the United States of America* 104:17123-17127.

913

914 Wong G, Audet J, Fernando L, Fausther-Bovendo H, Alimonti J B, Kobinger GP et al. (2014)  
915 Immunization with vesicular stomatitis virus vaccine expressing the Ebola glycoprotein  
916 provides sustained long-term protection in rodents. *Vaccine* 32:5722-5729.

917

918

919 **FIGURE LEGENDS**

920

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

924

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

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

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

935

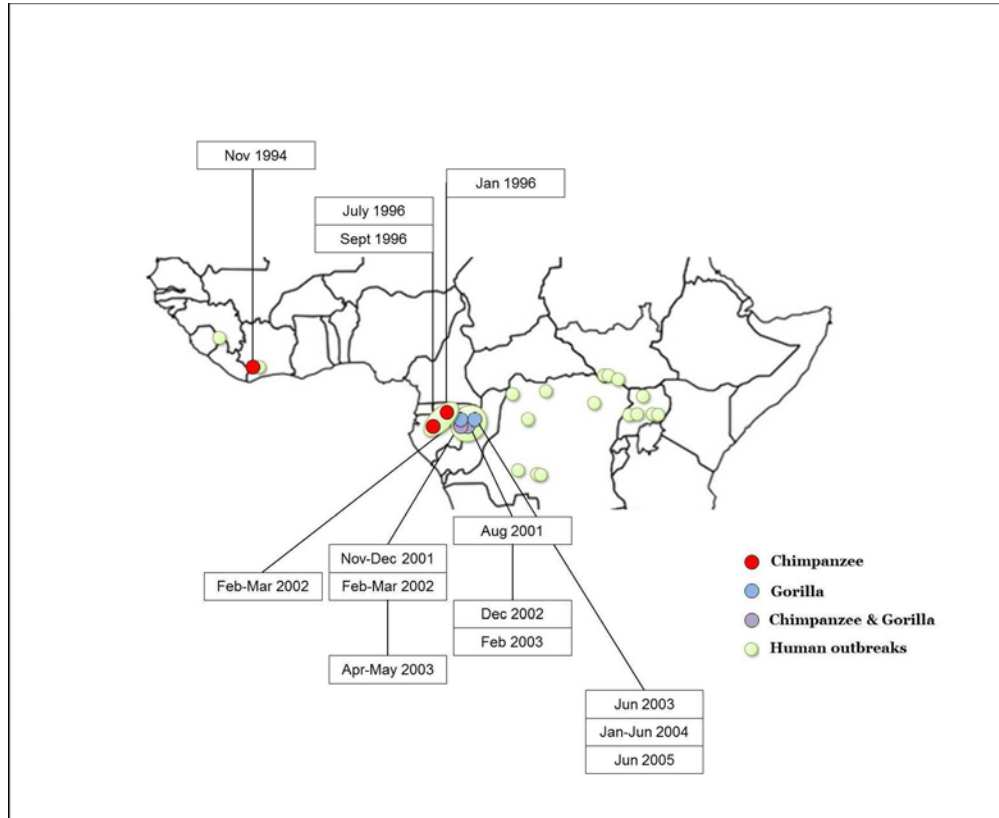


Fig. 1.

288x236mm (150 x 150 DPI)

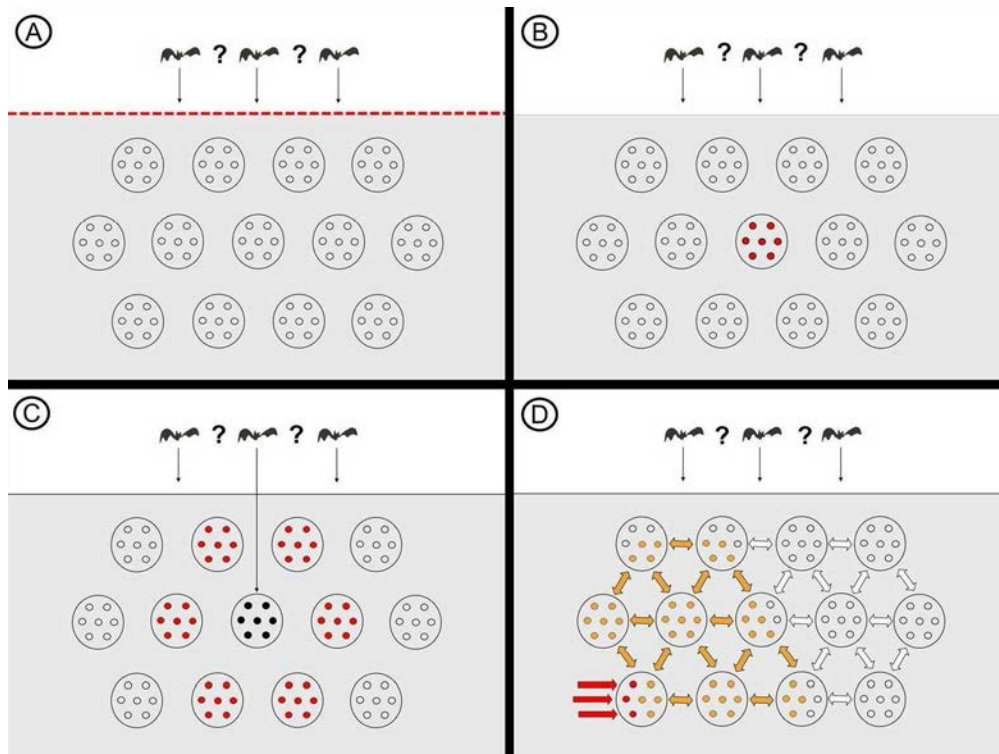


Fig. 2  
Fig. 2  
252x189mm (150 x 150 DPI)

Only