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Chlamydia-Related Bacteria in Free-Living and Captive Great Apes, Gabon

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To the Editor: Central Africa is the natural habitat for most of the world's gorillas and approximately one third of all chimpanzees. As a result of poaching, diseases, and habitat loss, the western lowland gorilla (*Gorilla gorilla gorilla*) and the central chimpanzee (*Pan troglodytes troglodytes*), both referred to as great apes, have been decreasing in numbers since 1970 and are now red-listed by the International Union for Conservation of Nature (*1*). Infectious diseases are major threats to apes in Africa. In addition to Ebola virus disease, a leading cause of death, the health of great apes is compromised by infections with *Bacillus anthracis*, *Staphylococcus aureus*, and *Plasmodium falciparum* (*1–4*). Chimpanzees and gorillas are closely related to humans and have similar anatomic, physiologic and immunologic features. Transmission of pathogens from humans to wildlife has been considered a major concern of tourism (*1*).

Except 1 report of bacteria of the order Chlamydiales in a fecal sample from a wild-living Congolese *P. troglodytes troglodytes* (*5*), nothing is known about the prevalence of Chlamydiales in great apes. Members of this order are obligate intracellular pathogens that have a unique biphasic life cycle. They infect a wide range of hosts and have major effects on animal and human health worldwide. Until 1993, *Chlamydiaceae* was the only known chlamydial family. However, the discovery of numerous *Chlamydia*-related bacteria species indicated a much broader diversity and host spectrum (*6*). To learn more about the prevalence of Chlamydiales in great apes, we analyzed samples from critically endangered western lowland gorillas and endangered central chimpanzees from Gabon.

We screened 25 samples (8 ocular, 4 vaginal, 7 penile, and 6 rectal swab specimens) obtained noninvasively during routine health checks from 12 apes in captivity. At the time of sampling, the animals were anesthetized and showed no evident signs of disease. All apes were born and reared in captivity at the Primatology Unit of the International Centre for Medical Research of Franceville (Franceville, Gabon) and lived in social groups of ≈10 animals.

We also analyzed feces from wild-living gorillas and chimpanzees, 10 samples from each species, collected in several remote forest areas of Gabon. All samples were collected according to international guidelines used at the

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International Centre for Medical Research of Franceville. For fecal samples obtained immediately after defecation, the outer layer was removed by using a sterile scalpel, and material from the inner part was frozen to avoid degradation and surface contamination.

Extracted DNA from swab specimens and feces was initially screened for *Chlamydiaceae* by using a 23S rRNA real-time PCR and primers Ch23S-F and Ch23S-R (*7*). A internal control amplification was performed with primers EGFP-1-F and EGFP-10-R, and *Chlamydia abortus* DNA was used to prepare a standard curve.

To detect other Chlamydiales, all samples were analyzed by using a broad-range, pan-Chlamydiales 16S rRNA real-time PCR, which had a sensitivity of 94% and showed no cross-amplification with DNA from other bacterial clades (*8*). Plasmid pCR2.1-TOPO (Invitrogen, Basel, Switzerland), which contained a portion of the 16S rRNA gene targeted by the pan-Chlamydiales 16S rRNA real-time PCR, was used to produce a standard curve. Samples with a cycle threshold <35 were sequenced (GATC Biotech AG, Konstanz, Germany), and results were analyzed by using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Purification, real-time PCR, sequencing PCR, and electrophoresis were performed in different laboratories to avoid DNA contamination.

The 16S rRNA real-time PCR and sequencing identified Chlamydiales of the non-*Chlamydiaceae* families in captive and free-living chimpanzees and gorillas. However, we did not identify species in the family *Chlamydiaceae* (Table). For captive great apes, BLAST analysis of 1 rectal (gorilla) and 1 penile (chimpanzee) sample showed 100% and 98% sequence identity, respectively, with *Waddlia chondrophila*. Furthermore, *Candidatus* Rhabdochlamydia sp. cvE88 was found in a vaginal swab specimen of 1 chimpanzee (99% sequence identity) and was still detectable in a second sample from the same site 1 month later. Among free-living apes, 3 of 10 chimpanzee samples were positive for Chlamydiales and showed 96%–99% identity with uncultured Chlamydiales CRG97. One fecal sample from a gorilla contained *W. chondrophila* (100% sequence identity). Chlamydiales detected in urogenital samples might have been acquired through smear infections. For omnivorous chimpanzees, Chlamydiales in fecal samples might have originated from ingestion of infected prey.

We detected members of the order Chlamydiales in great apes from Gabon. Our study not only identified a new chlamydial host but could also help to gain deeper insights into the evolution of Chlamydiales. The emerging pathogen *W. chondrophilia* has been implicated in human and bovine miscarriage and reported to be transmitted zoonotically or after exposure to freshwater amebae infected with *Chlamydia*-related bacteria (*9*,*10*). Further studies are required to determine the prevalence of Chlamydiales in primates and their potential for causing disease in great apes in Africa threatened with extinction.

Table. Analysis of 7 captive and free-living apes for *Chlamydia*-related bacteria by using real-time PCR and sequencing, Gabon*

Ape	Source	Species	Mean C_t	DNA copies/μL	Closest BLAST† match for 16S rRNA gene	Sequence identity, %	Fragment size, bp	E-value
Cola‡	Rectal swab	*Gorilla gorilla gorilla*	33.02	10.57	*Waddlia chondrophila* WSU 86–1044, complete sequence	100	230	1×10^{-115}
Cabinda‡	Penile swab	*Pan troglodytes troglodytes*	33.32	8.58	*W. chondrophila* WSU 86–1044, complete sequence	98	241	1×10^{-111}
Djela‡	Vaginal swab§	*P. troglodytes troglodytes*	29.34	122.41	*Candidatus* Rhabdochlamydia sp. cvE88, partial sequence	99	243	8×10^{-118}
1882¶	Feces	*P. troglodytes troglodytes*	34.29	8.24	Uncultured Chlamydiales CRG97, partial sequence	98	201	6×10^{-93}
1883¶	Feces	*P. troglodytes troglodytes*	31.90	43.55	Uncultured Chlamydiales CRG97, partial sequence	99	200	1×10^{-95}
1885¶	Feces	*P. troglodytes troglodytes*	31.16	73.41	Uncultured Chlamydiales CRG97, partial sequence	96	209	1×10^{-90}
Gab2130¶	Feces	*G. gorilla gorilla*	35.30	2.38	*W. chondrophila* WSU 86–1044, complete sequence	100	218	5×10^{-109}

*C_t, cycle threshold.
†http://blast.ncbi.nlm.nih.gov/Blast.cgi
‡Captive ape.
§A second vaginal swab specimen from the same chimpanzee that was collected 1 mo later still showed a positive result by real-time PCR, and sequencing indicated the presence of a *Candidatus* Rhabdochlamydia sp. cvE88.
¶Free-living ape.

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Schmallenberg Virus in Zoo Ruminants, France and the Netherlands

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To the Editor: Schmallenberg virus (SBV), a new orthobunyavirus of the family *Bunyaviridae*, emerged in August 2011 in northwestern Europe (*1*) and spread to most parts of Europe by *Culicoides* vectors (*2*). Most infections are asymptomatic in adult ruminants, yet fever, milk drop, and diarrhea have been reported (*1*). SBV is responsible for congenital malformations in newborn calves, lambs, and goat kids and has also been associated with abortions and early embryonic losses (*3*). The virus affects domestic livestock, but antibodies to SBV have also been found in free-ranging wild ruminants in several European countries (*3–6*) and in wild and exotic ruminants kept in captivity in the United Kingdom and in Austria (*3–5*). We carried out a study to investigate the exposure to SBV of wild and exotic ruminants born in Europe and kept in 1 zoological park in France and 1 in the Netherlands.

We tested 42 serum samples (from 39 animals) collected between 2011 and 2014 in the Safaripark Beekse Bergen (SPBB, Hilvarenbeek, the Netherlands) and 18 serum samples (from 15 animals) collected between 2013 and 2015 in the Ménagerie du Jardin des Plantes, Muséum National d'Histoire Naturelle (MJP, Paris, France). First, we determined the presence of SBV-specific antibodies in the samples by ELISA (ELISA ID Screen SBV Competition; ID Vet, Grabels, France) and by virus neutralization test (VNT) according to a protocol previously described (*7*). The 2 methods gave identical results except for 5 samples found negative by ELISA and positive by VNT. Thirty (55.6%) of 54 animals were found to be seropositive by VNT, which is regarded as the standard for SBV detection (Table). Antibodies to SBV were found in 11 (73.3%) of 15 animals from MJP and 19 (48.7%) of 39 animals from SPBB. Positive results were found in samples collected every year during 2011–2015; the earliest positive result was found in a sample collected in September 2011 (SPBB).

Several seropositive ruminants from MJP were either born in Paris or transferred to Paris from another park in Europe before 2010, which suggests that they were exposed to SBV in Paris. SBV antibodies were found in 3 consecutive samples collected in October 2011, September 2012, and March 2013 from a sable antelope (*Hippotragus niger niger*) in SPBB but also in 3 consecutive samples collected in October 2013, February 2014, and September 2014 in a bharal (*Pseudois nayaur*) from MJP. These data suggest that SBV antibodies can persist for ≥1 year in these 2 species.

We then performed SBV-specific quantitative reverse transcription PCR targeting the small segment (*8*) of the virus on every sample. One sample from an SBV seronegative