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 Chlamydi-ales in a fecal sample from a wild-living Congolese P. troglodytes troglodytes (5), nothing is known about the prevalence of Chlamydiiales 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 Chlamydiiales 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 during routine health checks from 10 animals.

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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-Chlamydiaceae 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-Chlamydiaceae 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 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 Chlamydiaceae and showed 96%–99% identity with uncultured Chlamydiaceae CRG97. One fecal sample from a gorilla contained W. chondrophila (100% sequence identity). Chlamydiaceae detected in urogenital samples might have been acquired through smear infections. For omnivorous chimpanzees, Chlamydiaceae in fecal samples might have originated from ingestion of infected prey.

We detected members of the order Chlamydiaceae 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 Chlamydiaceae. The emerging pathogen W. chondrophila 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 Chlamydiaceae 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<sub>t</sub> | DNA copies/μL | Closest BLAST† match                  | Sequence identity, % | Fragment size, bp | E-value  \\
|-----|-----------|---------------------|-------------------|---------------|---------------------------------------|----------------------|------------------|---------- |
| Cola‡| Rectal swab| Gorilla gorilla gorilla | 33.02             | 10.57         | Waddlia chondrophila                  | 100                  | 230              | 1 × 10<sup>-116</sup> |
| Cabinda‡ | Penile swab | Pan troglodytes troglodytes | 33.32             | 8.58          | W. chondrophila WSU 86–1044, complete sequence | 98                  | 241              | 1 × 10<sup>-111</sup> |
| Djela‡ | Vaginal swab§ | P. troglodytes troglodytes | 29.34             | 122.41        | Candidatus Rhabdochlamydia sp. cvE88, partial sequence | 99                  | 243              | 8 × 10<sup>-118</sup> |
| 1882¶ | Feces | P. troglodytes troglodytes | 34.29             | 8.24          | Uncultured Chlamydiaceae CRG97, partial sequence | 98                  | 201              | 6 × 10<sup>-93</sup> |
| 1883¶ | Feces | P. troglodytes troglodytes | 31.90             | 43.55         | Uncultured Chlamydiaceae CRG97, partial sequence | 99                  | 200              | 1 × 10<sup>-95</sup> |
| 1885¶ | Feces | P. troglodytes troglodytes | 31.16             | 73.41         | Uncultured Chlamydiaceae CRG97, partial sequence | 96                  | 209              | 1 × 10<sup>-90</sup> |
| Gab2130¶ | Feces | G. gorilla gorilla | 35.30             | 2.38          | W. chondrophila WSU 86–1044, complete sequence | 100                 | 218              | 5 × 10<sup>-109</sup> |

*C<sub>t</sub>, 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.
Schmallenberg Virus in Zoo Ruminants, France and the Netherlands

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To the Editor: Schmallenberg virus (SBV), a new orbivirus 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

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