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SHORT COMMUNICATIONS

EVIDENCE FOR THE PRESENCE OF *CHLAMYDIA* IN WILD MAMMALS OF THE SERENGETI

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Abstract

Only limited information is available on the presence of *Chlamydiaceae* in wildlife, a deficit that is particularly acute concerning the mammalian wildlife population in Africa. In a retrospective analysis of organ material from an earlier study on wild mammals from the Serengeti National Park, 521 samples from 54 animals of 14 different mammalian species were investigated. The presence of *Chlamydiaceae* was analyzed using molecular methods and immunohistochemistry. Chlamydial DNA was detected by real-time PCR in formalin-fixed and paraffin-embedded tissues from large ruminants (African buffaloes, *Syncerus caffer*, *n* = 4) and large canine predator (spotted hyena, *Crocuta crocuta*, *n* = 1). Microarray results revealed *C. abortus* in all cases, confirmed by sequencing of selected samples, and a mixed infection with *C. abortus* and *C. pneumoniae* in African buffalo (*Syncerus caffer*, *n* = 1). This study resulted in the first report of *Chlamydiaceae* in African wildlife of the Serengeti area.

**Keywords:** Chlamydia, Serengeti, wild mammalian species

Chlamydiae are gram-negative, obligate intracellular bacteria infecting a broad range of animal species. They are widespread in nature and can cause a variety of clinical symptoms, affecting ocular, pulmonary, genital, articular, and intestinal tissues. Chlamydial strains have traditionally been considered to be host-related, however, this specificity is becoming increasingly challenged (Pospischil et al., 2010).

Only limited information is available on the presence of *Chlamydiaceae* in wildlife worldwide, a lack of knowledge that is particularly acute for mammalian wildlife populations in Africa. In a serological survey, 58 serum samples from free-ranging mountain lions (*Felis concolor*) in California were negative for antibodies to
*Chlamydia (C.) psittaci* by complement fixation test and indirect fluorescent antibody assay (Paul-Murphy et al., 1994).

The aim of the present study was to re-investigate samples collected during a previous study on morbillivirus infections in the Serengeti National Park in Tanzania, Africa. Organ material from this study (Roelke-Parker et al., 1996) as well as samples from other wild mammalian species from the same region were investigated for the presence of *Chlamydia*.

A total of 521 organ samples (*n* = 521) from 54 animals belonging to 14 species were analyzed (Table 1). They were collected during a collaboration between the Tanzania National Park, Africa and the Institute of Veterinary Pathology in the Serengeti National Park during 1993 to 1995. The following organ tissues were tested: skin, adipose tissue, lymphatic organs, muscle (unspecified location), bone, heart, aorta, nasal cavity, lung, liver, urogenital tissues, gastrointestinal tissues, pancreas, adrenal gland, thyroid gland, salivary gland, tongue, tonsil, umbilical cord, brain, peripheral nerve, spinal cord and eye.

From formalin-fixed and paraffin-embedded material, sections of 30 - 60 µm were cut from each paraffin block and placed into a microcentrifuge tube (2 - 3 paraffin blocks per animal were randomly pooled) and processed as previously described (Borel et al., 2006). DNA for PCR analysis was extracted from the tissue pellet using a commercial DNA extraction kit (DNeasy Tissue kit, Qiagen, Hilden, Germany).

All samples (*n* = 521) were examined on an ABI 7500 Fast real-time PCR system instrument (Applied Biosystems, Foster City, California, USA) using the 23S-based *Chlamydiaceae* family-specific real-time PCR as described previously (Ehricht et al., 2006). The methodology yields a 111-bp product specific for members of the
family *Chlamydiaceae*. A cycle threshold (Ct value) of < 38.00 was considered as positive and Ct values above 38 were interpreted as questionable. All samples were tested at least in duplicate.

The samples with at least one positive Ct value were examined using the species-specific 23S ArrayTube (AT) microarray assay (Alere, Jena, Germany) as described by Borel et al. (2008).

Samples positive for *Chlamydiaceae* by real-time PCR, but negative by the AT test for chlamydial species identification were further examined using the primer pair 16S-IGF (5′-GAT GAG GCA TGC AAG TCG AAC G-3′) and 16S-IGR (5′-CCA GTG TTG GCG GTC AAT CTC TC-3′) targeting the *Chlamydiales*-specific 298-bp 16S rRNA signature sequence, as previously described (Everett et al., 1999). Sequencing of 16S rRNA PCR products was performed in collaboration with the sequencing service of the University of Zurich with an Applied Biosystems 3130xl Genetic Analyzer (Applied Biosystems). The sequences obtained were identified by BLAST-n searching (Altschul et al., 1997) of the sequences available in GenBank using the BLAST server from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST).

Paraffin sections from real-time PCR positive animals were investigated for the presence of chlamydial antigen by using a *Chlamydiaceae* family-specific mouse monoclonal antibody directed against the chlamydial lipopolysaccharide (LPS, Clone ACI-P, Progen, Heidelberg, Germany) as previously described (Borel et al., 2006). Intestinal tissue from gnotobiotic piglets (*Sus scrofa*) experimentally infected with porcine *C. suis* strain S45 was used as a positive control (Guscetti et al., 2000).

Chlamydial DNA was detected by real-time PCR from formalin-fixed and paraffin-embedded tissues of five animals in the Serengeti. Affected animal species
were large ruminants (African buffaloes, *Syncercus caffer, n = 4*) and large canine predator (spotted hyena, *Crocuta crocuta, n = 1*). Details of animals positive for *Chlamydiaceae* are presented in Table 2. Microarray analysis revealed *C. abortus* in all cases, confirmed in selected samples by sequencing of the *Chlamydiales*-specific signature sequence (Everett et al., 1999), and a mixed infection with *C. abortus* and *C. pneumoniae* in one African buffalo (*Syncercus caffer, no. 3*). *Chlamydia abortus*-positive samples were obtained from paraffin blocks including different organs whereas the mixed infection with *C. abortus* and *C. pneumoniae* was detected in a pooled sample containing small intestine, heart, adrenal gland and lymph node.

Immunohistochemistry for *Chlamydiaceae* was positive in the small intestine and lymph node of the spotted hyena (*Crocuta crocuta*) as well as in liver, kidney and lung in three African buffaloes (*Syncercus caffer, Table 2*). All organ samples of African buffalo (*Syncercus caffer, no. 3*) were negative by immunohistochemistry for *Chlamydiaceae* (Table 2). Comparing the methods of detection, there was a correlation between the relatively low amount of antigen detected in the tissues by immunohistochemistry (a few positively labeled cells in four animals) and high Ct values in the real-time PCR. This is likely to be an indication of a low chlamydial load in the individual animal.

Pathological findings were available for several animals. The *C. abortus*-positive spotted hyena (*Crocuta crocuta*) was positive for canine distemper virus and died due to a sepsis (data not shown). African buffalo (*Syncercus caffer*) no. 1 had a lung emphysema but cause of death remained unknown. African buffalo (*Syncercus caffer*) no. 2 suffered from an interstitial pneumonia, enteritis, hepatitis and dermatitis. Animal no. 4 (newborn of buffalo no. 3) showed pathologic findings in
lung, spleen, heart and intestine (data not shown). All other animals \((n = 49)\) were negative for *Chlamydiaceae* by real-time PCR.

This is the first report on the presence of *Chlamydiaceae* (*C. abortus* and *C. pneumoniae*) in African wildlife (*Syncercus caffer, Crocuta crocuta*) in the Serengeti area. *Chlamydia abortus* is endemic among small ruminants worldwide but is diagnosed in *Bovidae* as well (Pospischil et al., 2010) and has generally been associated with abortion (Borel et al., 2006). In North Africa, the occurrence of *C. abortus* in 14 sheep (*Ovis aries*) and 23 goat (*Capra aegagrus hircus*) flocks, respectively was reported using CFT and ELISA in Tunisia (Benkirane et al., 1990; Rekiki et al., 2002). A serologic study from Namibia (Apel et al., 1989) indicates that *C. abortus* infections were prevalent in all the geographical regions that were tested in this country. To our knowledge, there is only one other report on the isolation of *Chlamydia* from a free living African buffalo (*Syncercus caffer*) in Botswana (Rowe et al., 1978). According to the methods available at that time the isolate was characterized as *C. psittaci* (Rowe et al., 1978). Gupta et al. (1976), however, report the isolation of *Chlamydia* from a water buffalo calf (*Bubalus bubalis*) in India. We now report for the first time an infection with *C. abortus* in four African buffaloes (*Syncercus caffer*) in the Serengeti. It can be speculated that *C. abortus* is present in cattle (*Bos primigenius*) in the Serengeti area and African buffaloes (*Syncercus caffer*) may occasionally come in contact with Massai cattle herds seasonally present in the area and thus a transmission of infectious agents like *Chlamydia* could occur.

Spotted hyenas (*Crocuta crocuta*) as predators feed, amongst other sources, on carcasses of fallen buffaloes and might possibly get in contact with *Chlamydia* by this means. In our study, we were able to detect *C. abortus* in one spotted hyena
(Crocuta crocuta). This chlamydial species is most likely acquired by carcass consumption.

In one Buffalo (Syncerus caffer), a mixed infection of C. abortus and C. pneumoniae was detected. Mixed infections of chlamydial species in vivo are rare but exist. To detect those infections, however, methods like the ArrayTube microarray are necessary (Borel et al., 2008; Holzwarth et al., 2011). The host range of C. pneumoniae was originally believed to be restricted to humans, but was lately expanded to horses (Equus ferus caballus), koalas (Phascolarctos cinereus), reptiles (Reptilia) and amphibians (Amphibia) (Bodetti et al., 2002). In wild ruminants, C. pneumoniae has been recently detected in ibex (Capra ibex) and chamois (Rupicapra r. rupicapra) (Holzwarth et al., 2011).

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