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Evidence for *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 mammalian wildlife in Africa. In a retrospective analysis of organ material from an earlier study on wild mammals from the Seregenti National Park, 521 samples from 54 animals of 14 mammalian species were investigated. The presence of *Chlamydiaceae* was analyzed using molecular methods and immunohistochemistry. Chlamydial DNA was detected by real-time polymerase chain reaction in formalin-fixed and paraffin-embedded tissues from large ruminants (African buffaloes, *Syncerus caffer*, $n=4$) and a large predator (spotted hyena, *Crocuta crocuta*, $n=1$). Microarray results revealed *Chlamydia abortus* in all cases, confirmed by sequencing of selected samples, and a mixed infection with *Chlamydia abortus* and *Chlamydia pneumoniae* in an African buffalo. This is the first report of *Chlamydiaceae* in African wildlife of the Serengeti area.

Key words: Africa, *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 traditionally have been considered 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 serologic survey, 58 serum samples from free-ranging mountain lions (*Felis concolor*) in California, USA, were negative for antibodies to *Chlamydia psittaci* by complement fixation test and indirect fluorescent antibody assay (Paul-Murphy et al., 1994).

Our aim was to reinvestigate 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) and samples from other wild mammalian species from the same region were tested for *Chlamydia*.

We analyzed 521 organ samples ($n=521$) from 54 animals of 14 species (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. Organ tissues tested were: 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 polymerase chain reaction (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 rRNA-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

TABLE 1. Details of wild mammalian species ($n=54$) sampled in the Serengeti National Park examined for *Chlamydia*, 1993–1995.

Order	Family	Species	*n*
Carnivora	Felidae	Lion (*Pathera leo*)	17
Perissodactyla	Equidae	Zebra (*Equus zebra*)	9
Carnivora	Hyenidae	Spotted hyena (*Crocuta crocuta*)	7
Artiodactyla	Bovidae	African buffalo (*Syncerus caffer*)	5
Carnivora	Felidae	Cheetah (*Acinonyx jubatus*)	3
Artiodactyla	Bovidae	Thomson's gazelle (*Eudorcas thomsonii*)	3
Proboscidea	Elephantidae	African elephant (*Loxodonta africana*)	2
Carnivora	Canidae	Jackal (*Canis aureus bea*)	2
Carnivora	Canidae	Bat-eared fox (*Otocyon megalotis*)	1
Carnivora	Canidae	Dog (*Canis lupus*)	1
Carnivora	Herpestidae	Mongoose (*Herpestes naso*)	1
Artiodactyla	Giraffidae	Giraffe (*Giraffa camelopardalis*)	1
Artiodactyla	Bovidae	Impala (*Aepyceros melampus*)	1
Artiodactyla	Bovidae	Wildebeest (*Connochaetes taurinus*)	1

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 rRNA 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, Switzerland, with an Applied Biosystems 3130×l Genetic Analyzer (Applied Biosystems). 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 chlamydial antigen 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, *Syncerus caffer*, $n=4$) and a large 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 (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

TABLE 2. Details of *Chlamydiaceae*-positive animals ($n=5$) by real-time polymerase chain reaction (PCR) for *Chlamydiaceae*, ArrayTube chlamydial species identification, 16S rRNA PCR and immunohistochemistry (IHC).

Animal no. pool	Organs[a]	Real-time PCR *Chlamydiaceae* (mean Ct value)[b]	ArrayTube microarray identification assay[c]	16S rRNA PCR Homology (%)[c]	IHC
African buffalo 1: 1.5-yr-old male					
A	skin, lung	positive (37.9)	*Chlamydia abortus*	ND	negative
B	lung, spleen, kidney	questionable (38.3)	*C. abortus*	ND	negative
C	heart, liver, ln	negative	ND	ND	positive[d]
D	heart	positive (33.8)	*C. abortus*	ND	negative
African buffalo 2: 6-yr-old male					
A	skin	positive (33.5)	*C. abortus*	ND	negative
B	spleen, liver, abomasum	positive (37.3)	*C. abortus*	ND	negative
C	rumen, heart, testis, sm int	positive (35.6)	*C. abortus*	ND	negative
D	kidney, lung, epididymis	positive (35.2)	*C. abortus*	ND	positive[e]
E	heart, sm int	positive (35.1)	*C. abortus*	ND	negative
F	heart, adrenal gland, ln	questionable (38.2)	negative	negative	negative
African buffalo 3: 4-yr-old female					
A	ovary, pancreas, ab, sg	positive (35.5)	*C. abortus*	ND	negative
B	mamma, uterus, ln, la int	positive (35.8)	*C. abortus*	ND	negative
C	rumen, lung, spleen	positive (35.5)	*C. abortus*	ND	negative
D	heart, ln, sm int, adrenal gland	positive (35.6)	*C. abortus*/*C. pneumoniae*	ND	negative
E	heart, liver, abomasum	positive (35.4)	*C. abortus*	ND	negative
F	heart, kidney, sm int	positive (34)	*C. abortus*	ND	negative
African buffalo 4: newborn female					
A	liver, kidney, spleen, placenta	positive (35.2)	*C. abortus*	ND	negative
B	lung, heart, forestomachs	positive (37.1)	*C. abortus*	ND	positive[f]
C	psalter, sm int, ln	positive (35.6)	*C. abortus*	ND	negative
Spotted hyena: 24-wk-old female					
A	lung, kidney, spleen	positive (34.5)	*C. abortus*	ND	negative
B	liver, thyroidea, muscle, ln	positive (36.3)	negative	*C. abortus* (98%)	negative
C	pancreas, heart, ln,	questionable (39.13)	*C. abortus*	ND	negative
D	brain, ln, sm int, la int	positive (37.1)	negative	*C. abortus* (100%)	positive[g]
E	brain, spleen	positive (34.7)	*C. abortus*	ND	negative

[a] ab = abomasum; la int = large intestine; ln = lymph node; sg = salivary gland; sm int = small intestine.

[b] Ct = cycle treshold.

[c] ND = not done.

[d] liver.

[e] kidney.

[f] lung.

[g] small intestine, lymph node.

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 as well as in liver, kidney, and lung in three African buffaloes (Table 2). All organ samples of African buffalo (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.

Pathologic findings were available for several animals. The *C. abortus*-positive spotted hyena was positive for canine distemper virus and died due to a sepsis (data not shown). African buffalo no. 1 had a lung emphysema but cause of death remained unknown. African buffalo 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 (*Syncerus 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 generally has been associated with abortion (Shewen, 1980). In North Africa, the occurrence of *C. abortus* in 14 sheep (*Ovis aries)* and 23 goat (*Capra aegagrus hircus*) flocks, respectively, was reported using complement fixation test (CFT) and enzyme-linked immunosorbent assay (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 geographic regions that were tested in that country. To our knowledge, there is only one other report of the isolation of *Chlamydia* from a free-living African buffalo 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 bubalus*) in India. We now report for the first time an infection with *C. abortus* in four African buffaloes in the Serengeti. It can be speculated that *C. abortus* is present in cattle (*Bos primigenius*) in the Serengeti area, and African buffaloes occasionally might come in contact with Massai cattle herds seasonally present in the area, and thus a transmission of infectious agents such as *Chlamydia* could occur.

Spotted hyenas as predators feed, among other sources, on carcasses of fallen buffaloes and might possibly contact *Chlamydia* this way. In our study, we detected *C. abortus* in one spotted hyena. This chlamydial species is most likely acquired by carcass consumption.

In one buffalo, a mixed infection of *C. abortus* and *C. pneumoniae* was detected. Mixed infections of chlamydial species in vivo are rare. 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 lately has been 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). Our results raise concerns over the origin and impact of chlamydial infections on African mammals and warrant further investigation.

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