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# Novel Chlamydia species isolated from snakes are temperature-sensitive and exhibit decreased susceptibility to azithromycin

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Correction to: Scientific Reports https://doi.org/10.1038/s41598-018-23897-z, published online 04 April 2018

The original version of this Article contains errors.

The description of *Chlamydia serpentis* sp.nov and *Chlamydia poikilotherma* sp. nov was incomplete.

Those species are validated herein:

Description of Chlamydia. serpentis sp. nov. (ser.pen'tis. L. gen. n. serpentum, of the snake)

C. serpentis strains occur in snakes belonging to the families Colubridae and Viperidae and were isolated from captive asymptomatic Patherophis guttatus and Atheris squamigera. The presence of the agent in other snake species as well as in free-ranging snakes and even other reptiles seems possible, but has yet to be investigated. C. serpentis can be recovered from choanal and cloacal swabs and might be also detected in inner organs of infected reptiles. The natural route of transmission and potential reservoirs are unknown to date. The carrier snakes were clinically asymptomatic, but a facultative pathogenic role has to be considered in concert with other bacterial or viral infections, or induced by stress due to capture and transportation, high-density farming and hibernation<sup>12</sup>. The potential for zoonotic infection of humans, in particular snake owners, is unknown.

C. serpentis can be grown in LLC-MK2 cells, a rhesus monkey epithelial kidney cell line, which has been successfully used to isolate C. suis strains from fecal swab samples<sup>24</sup> and is able to survive and replicate at lower temperature such as 28 °C and 12 °C. The replication of C. serpentis is enhanced by adding cycloheximide after the infection to block de novo host protein synthesis similar as shown for C. pneumoniae K6. Shape, size and distribution of inclusions including their production of infectious EBs measured as IFU per mL at 32 and 48 hpi resemble those seen in C. pneumoniae-infected LLC-MK2 cells. Size of inclusions and productivity of C. serpentis at 28 °C is diminished compared to 37 °C in line with C. pneumoniae K6 in this study. By TEM, the typical bi-phasic developmental cycle can be observed for C. serpentis including EBs and RBs comparable in size and morphology to C. pneumoniae. C. serpentis is susceptible to tetracycline and moxifloxacin but has an intermediate sensitivity of azithromycin (ranging from 2 to more than 4 µg/mL).

The type strain H15-1957-10C<sup>T</sup> (DSM 106151, CSUR Q5983) and reference strain H15-1957-3C (DSM 106152, CSUR Q5983) have been deposited at the DSMZ (Deutsche Sammlung von Mikrorganismen und Zellkulturen GmbH, Braunschweig, Germany) and at CSUR (Collection de Souches de l' Unité Rickettsies WDCM 875, Marseille, France).

### Description of Chlamydia. poikilotherma sp. nov.

(poi.ki.lo.ther'ma. N.L. fem. adj. poikilotherma poikilothermic)

Two highly similar strains of this species occurred in a captive *Pantheropis guttatus* belonging to the family *Colubridae*. The agent can be recovered from choanal and cloacal swab and is possibly also present in other snake families, captive or free-ranging. A pathogenic potential cannot be differentiated from the cause of death (salmonellosis) in the actual case. The mode of transmission and zoonotic potential are unknown. Like other *Chlamy-diaceae* species, *C. poikilotherma* can be isolated and grown in cell culture but requires lower temperatures such as 28 °C. Isolation at 37 °C is less successful, growth curves (Supplementary Figure S1) over time show the ability of *C. poikilotherma* to replicate at 37 °C but inclusions are significantly smaller and morphologically similar to ABs. The replication of *C. poikilotherma* is not enhanced by adding cycloheximide. It grows better in the absence of cycloheximide regardless of the temperature (28 °C, 37 °C). By IF, the inclusion morphology at 28 °C is heterogenous and inclusions tend to grow around host cell nuclei. The ultrastructural features of *C. poikilotherma* display EBs and RBs replicating by binary fission.

C. poikilotherma is susceptible to tetracycline and moxifloxacin but behaves intermediate to resistant to azithromycin (ranging from 2–4  $\mu$ g/mL). The type strain S15-834K<sup>T</sup> (DSM 106149, CSUR Q6003) and reference strain S15-834C (DSM 106150, CSUR Q6004) have been deposited at the DSMZ (Deutsche Sammlung von Mikrorganismen und Zellkulturen GmbH, Braunschweig, Germany) and at CSUR (Collection de Souches de l' Unité Rickettsies WDCM 875, Marseille, France).

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