Parasitology International 65 (2016) 367–370

Contents lists available at ScienceDirect

Parasitology International

journal homepage: www.elsevier.com/locate/parint

Short communication

Strongyloides infections of humans and great apes in Dzanga-Sangha Protected Areas, Central African Republic and in degraded forest fragments in Bulindi, Uganda

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ARTICLE INFO

Article history:
Received 18 December 2015
Received in revised form 10 April 2016
Accepted 10 May 2016
Available online 11 May 2016

Keywords:
Chimpanzee
Gorilla
Human
Strongyloides
Transmission
HVR-IV
Cox1

ABSTRACT

DNA sequence analysis was carried out on *Strongyloides* spp. larvae obtained from fecal samples of local humans, a wild western lowland gorilla (*Gorilla gorilla gorilla*) and a central chimpanzee (*Pan troglodytes troglodytes*) inhabiting Dzanga-Sangha Protected Areas (DSPA), Central African Republic, and eastern chimpanzees (*Pan troglodytes schweinfurthii*) living in degraded forest fragments on farmland in Bulindi, Uganda. From humans, both *Strongyloides fuelleborni* and *Strongyloides stercoralis* were recorded, though the former was predominant. Only *S. fuelleborni* was present in the great apes in both areas. Phylogenetic analysis of partial mtDNA cytochrome *c* oxidase subunit 1 gene (*Cox1*) and comparison of 18S rDNA hyper variable region IV (HVR-IV) sequences implied that in DSPA *S. fuelleborni* populations in humans differ from those in the nonhuman great apes.

Wild great apes and other non-human Old World primates harbor *Strongyloides* infections with a high prevalence [e.g. [1–4]]. Although it is assumed that most infections are caused by *S. fuelleborni*, mixed infections with *S. stercoralis* and *S. fuelleborni* have been reported from wild chimpanzees in Tanzania [5], suggesting that concomitant infections may not be uncommon. Human infection with *S. fuelleborni* was first recorded in Zimbabwe [1], and subsequently proved to be distributed widely in sub-Saharan countries, with an especially high prevalence in some groups inhabiting tropical forest areas, including Central African Republic (CAR), Cameroon and Ethiopia [6,7]. It remains unclear whether *Strongyloides* in humans are identical to or distinct from those infecting great apes and other non-human primates [8,9]. Recent molecular analysis found that dog-parasitic isolates of *S. stercoralis* formed a distinct phylogenetic clade from those parasitic in humans and apes [5]. Here, we analyzed *Strongyloides* DNA sequences originating from humans and wild great apes co-inhabiting the rainforest ecosystem of Dzanga-Sangha Protected Areas (DSPA), CAR [10] and from a population of eastern chimpanzees living in exceptionally close proximity to rural farmers in Bulindi, Uganda [11], to evaluate the zoonotic potential of these helminths.

As part of an ongoing health monitoring program, in 2011 fresh fecal samples were collected non-invasively from a wild western lowland

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http://dx.doi.org/10.1016/j.parint.2016.05.004
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Table 1
Number of *Strongyloides* larvae analyzed: Dzanga-Sangha Protected Areas (DSPA), Central African Republic and Bulindi, Uganda. Larvae used for mixed DNA extraction are shown in parentheses.

Locality	Host	No. fecal cultures	Total no. of larvae	Both HVR-IV and *Cox1*	HVR-IV only	*Cox1* only
DSPA	Eco-guard	1	3	1	1	1
	Trackers	4	8	3	(2)	3
	Gorilla	1	2	2		
	Chimpanzee	1	2	2		
Bulindi	Chimpanzees	5	14	4[a]	6 + (4)	

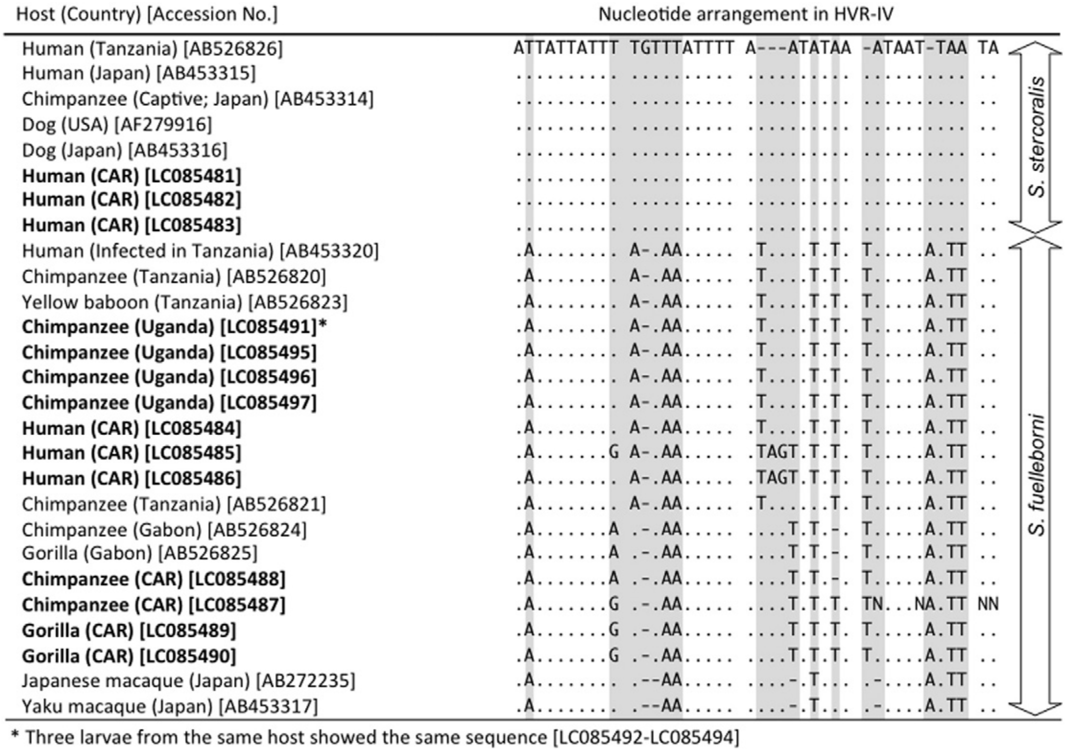
[a] Only shorter sequences of *Cox1* were obtained.

gorilla (*Gorilla gorilla gorilla*) and a central chimpanzee (*Pan troglodytes troglodytes*) in the Dzanga Sector of the Dzanga-Ndoki National Park of DSPA, south-western CAR (2°55′N, 16°20′E), alongside human fecal samples from local BaAka gorilla trackers and a Bantu Park eco-guard. Samples were collected also from eastern chimpanzees (*Pan troglodytes schweinfurthii*) in Bulindi (1°28′N, 31°28′E), Hoima District, western Uganda in 2013. Filariform larvae were raised using modified Harada-Mori filter-paper cultures in the field [12]. After 14 days, the larvae were fixed in 96 or ≥99% ethanol, and later transported to the laboratory, where *Strongyloides* larvae were selected morphologically under a stereomicroscope for DNA analysis. DNA was extracted from single larvae with the exception of two DNA samples, which were prepared from multiple larvae (two and four, respectively) developed in the same coprocultures (Table 1). DNA extraction, amplification and sequencing followed the protocol described in [10]. The primer sets used for amplification and sequencing of 18S rDNA hyper variable region IV (HVR-IV), which constitutes part of the V7 domain in SSU rRNA, and partial mtDNA cytochrome *c* oxidase subunit 1 gene (*Cox1*) of *Strongyloides* were SSU24HF 5′-AGAGGTGAAATTCGTGGACC-3′ (forward) and 18SPC 5′-ACGGGCGGTGTGTRC-3′ (reverse) [13], and StrCoxAfrF 5′-GTGGTTTTGGTAATTGAATGGTT-3′ (foward) and JB4.5 5′-TAAAGAAAGAACATAATGAAAATG-3′ (reverse) [5], respectively. Phylogenetic analysis of *Cox1* sequences was performed using MEGA5 (v. 5.03) [14].

For DSPA samples, both HVR-IV and partial *Cox1* with 714 base pairs (bp) were successfully sequenced for all larvae originating from the apes but only some from humans (Table 1). Amplification of *Cox1* from larvae from the Bulindi chimpanzees was problematic, and only 216, 503, 551 and 600 bp in 5′ side were sequenced (Table 1). The HVR-IV and *Cox1* sequences of DSPA samples divided the larvae into two groups corresponding to *S. stercoralis* and *S. fuelleborni*, while all of the Bulindi samples belonged to *S. fuelleborni* (Figs. 1, 2). All larvae from the eco-guard and one larva from a tracker belonged to *S. stercoralis*. Remaining larvae from both trackers and DSPA great apes belonged to *S. fuelleborni*. These disparities might reflect differences in occupations, life styles and sanitary conditions between BaAka and Bantu people.

The *S. stercoralis* nucleotide arrangements of HVR-IV were identical with those from various host species from different localities around the world (Fig. 1) [5,13,15]. On the contrary, the *S. fuelleborni* larvae nucleotide arrangements of HVR-IV were variable. Among the three genotypes found in the trackers in DSPA, one [LC085484] was identical with all HVR-IV sequences found in the Bulindi chimpanzee samples [LC085491-LC085497], and also with those previously recorded from a wild chimpanzee [AB526820], a yellow baboon [AB526822] and a researcher [AB453320] working with wild chimpanzees in Mahale Mountains NP, Tanzania [5]; the other two [LC085485, LC085486] differed from known genotypes of *S. fuelleborni* (Fig. 1). HVR-IV of the *S. fuelleborni* larvae from the DSPA chimpanzee [LC085488] was identical with those reported from a chimpanzee and a lowland gorilla in Gabon [AB526824; AB526825], while the HVR-IV of the *S. fuelleborni* larvae from the DSPA gorilla [LC085489, LC085490] was a new genotype (Fig. 1).

A nucleotide substitution from guanine to adenine resulted in an amino acid change from valine to isoleucine in one haplotype [LC085498] found in *Cox1* of *S. stercoralis* larva from the tracker, while three other substitutions found among the three isolates were



Host (Country) [Accession No.]	Nucleotide arrangement in HVR-IV	
Human (Tanzania) [AB526826]	ATTATTATTT TGTTTATTTT A---ATATAA -ATAAT-TAA TA	*S. stercoralis*
Human (Japan) [AB453315]	....	
Chimpanzee (Captive; Japan) [AB453314]	....	
Dog (USA) [AF279916]	....	
Dog (Japan) [AB453316]	....	
Human (CAR) [LC085481]	....	
Human (CAR) [LC085482]	....	
Human (CAR) [LC085483]	....	
Human (Infected in Tanzania) [AB453320]	.A........ A-.AA..... .T....T.T. T.....A.TT ..	*S. fuelleborni*
Chimpanzee (Tanzania) [AB526820]	.A........ A-.AA..... .T....T.T. T.....A.TT ..	
Yellow baboon (Tanzania) [AB526823]	.A........ A-.AA..... .T....T.T. T.....A.TT ..	
Chimpanzee (Uganda) [LC085491]*	.A........ A-.AA..... .T....T.T. T.....A.TT ..	
Chimpanzee (Uganda) [LC085495]	.A........ A-.AA..... .T....T.T. T.....A.TT ..	
Chimpanzee (Uganda) [LC085496]	.A........ A-.AA..... .T....T.T. T.....A.TT ..	
Chimpanzee (Uganda) [LC085497]	.A........ A-.AA..... .T....T.T. T.....A.TT ..	
Human (CAR) [LC085484]	.A........ A-.AA..... .T....T.T. T.....A.TT ..	
Human (CAR) [LC085485]	.A.......G A-.AA..... .TAGT.T.T. T.....A.TT ..	
Human (CAR) [LC085486]	.A........ A-.AA..... .TAGT.T.T. T.....A.TT ..	
Chimpanzee (Tanzania) [AB526821]	.A........ A-.AA..... .T......T. T.....A.TT ..	
Chimpanzee (Gabon) [AB526824]	.A.......A .-.AA..... ....T.T.-. T.....A.TT ..	
Gorilla (Gabon) [AB526825]	.A.......A .-.AA..... ....T.T.-. T.....A.TT ..	
Chimpanzee (CAR) [LC085488]	.A.......A .-.AA..... ....T.T.-. T.....A.TT ..	
Chimpanzee (CAR) [LC085487]	.A.......G .-.AA..... ....T.T.T. TN...NA.TT NN	
Gorilla (CAR) [LC085489]	.A.......G .-.AA..... ....T.T.T. T.....A.TT ..	
Gorilla (CAR) [LC085490]	.A.......G .-.AA..... ....T.T.T. T.....A.TT ..	
Japanese macaque (Japan) [AB272235]	.A........ .--AA..... ....-.T... .-....A.TT ..	
Yaku macaque (Japan) [AB453317]	.A........ .--AA..... ....-.T... .-....A.TT ..	

* Three larvae from the same host showed the same sequence [LC085492-LC085494]

Fig. 1. Comparison of nucleotide arrangements in 18S rDNA HVR-IV of *Strongyloides stercoralis* and *S. fuelleborni*. Alignment was made using ClustalW; dots represents the same nucleotide with AB526826; — indicates a gap. Positions with substitutions and/or indels are shaded. The newly sequenced materials are boldfaced.