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ORIGINAL PAPER

Accipiter hawks (*Accipitridae*) confirmed as definitive hosts of *Sarcocystis turdusi*, *Sarcocystis cornixi* and *Sarcocystis* sp. ex *Phalacrocorax carbo*

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Abstract *Sarcocystis* is a large genus of protozoan parasites with complex heteroxenous life cycles. For many species, either the intermediate or the definitive host is still unknown. In this study, 116 *Accipiter* hawks (Eurasian sparrowhawks and northern goshawks) were investigated for the presence of *Sarcocystis* spp. in their intestinal tract or their faeces. To gain a wide distribution, samples were collected throughout Germany within 2 years. It was possible to detect *Sarcocystis*-like oocysts in 65 samples. Sequencing of the ITS region or species-specific PCR identified 33 samples as *Sarcocystis turdusi/Sarcocystis* sp. ex *A. nisus* (18), *Sarcocystis calchasi* (6), *Sarcocystis columbae* (3), *Sarcocystis cornixi* (3) *and Sarcocystis* sp. ex *Phalacrocorax carbo* (3). Besides the known infestation with *S. columbae*, *S.* sp. ex *A. nisus* and *S. calchasi* the *Accipiter* hawks were thereby confirmed as definitive host of *S. turdusi*, *S. cornixi* and *S.* sp. ex *Phalacrocorax carbo* for the first time.

Keywords Avian *Sarcocystis* species · *Accipiter* hawks · Definitive host · Amplification of ITS region

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Introduction

Sarcocystis spp. are apicomplexan parasites with an obligatory two-host life cycle, consisting of carnivore/omnivore species as definitive hosts and herbivore/omnivore prey species as intermediate hosts (Mehlhorn and Heydorn 1978; Dubey et al. 1989). Previously, northern goshawks (*Accipiter gentilis*) and Eurasian sparrowhawks (*Accipiter n. nisus*) have been described as definitive hosts of *Sarcocystis calchasi*, *Sarcocystis columbae*, *Isospora buteonis* and *Sarcocystis accipiter sp. nisus* with avian intermediate hosts (Mehlhorn and Heydorn 1978; Svobodova 1996; Olias et al. 2011). However, other avian species, which belong to the prey spectrum of *Accipiter* hawks, such as the common blackbird (*Turdus merula*) or *Corvidae* species, were tested positive for *Sarcocystis* species which had not been identified in *Accipiter* hawks yet (Kutkiene et al. 2009, 2012). This study investigated the occurrence of different *Sarcocystis* spp. in samples of *Accipiter* hawks throughout Germany and whether the life cycles of *Sarcocystis* species with so far unknown definitive hosts could be concluded.

Material and methods

Sample collection

In the period of 2012 to 2014, a total of 116 samples from free-ranging *Accipiter* hawks were collected throughout Germany from veterinary clinics and rescue centres. These samples consisted of 61 carcasses, 16 intestines and 8 faecal samples from Eurasian sparrowhawks and 14 carcasses, 9 intestines and 8 faecal samples of northern goshawks. The intestines were sampled for DNA extraction at two locations of the small intestines and at one location in the large intestine. Additionally, three mucosal scrapings for light microscopic examination were taken at the same locations. From faecal samples, DNA was extracted in 200-mg portions whereas the remaining material was floated in saturated sodium chloride (specific gravity 1.2).

Light microscopy

Mucosal scrapings and faecal float were examined at ×400 magnification for the presence of protozoan organisms. Those samples with oocysts typical for *Sarcocystidae* were selected for further investigations.

Semi-nested polymerase chain reaction specific for *Sarcocystis calchasi*

DNA was extracted (DNeasy Blood & Tissue Kit/Stool Kit; Qiagen, Hilden, Germany) from intestinal and faecal samples. DNA concentration was measured (NanoDrop 2000c Spectrophotometer; Thermo Fisher Scientific, Wilmington, DE, USA) and, if necessary, diluted to <5 ng/μl. DNAse-free water was included as negative control, the DNA extracted from the Berlin strain of *S. calchasi* was used as positive control (Olias et al. 2011). A semi-nested polymerase chain reaction specific for *S. calchasi* was performed (Maier et al. 2014). All samples positive for *S. calchasi*-specific DNA were excluded from further investigations.

Amplification and sequencing of the ITS-1 region

The amplification of the ITS-1 region was performed with samples which contained *Sarcocystis*-like oocysts but were negative for *S. calchasi*-specific DNA. PCR was conducted as described previously with primers ITS-5/ITS-2 (White et al. 1990). Amplicons of the predicted size (approximately 840 bp) were extracted from the agarose gel, purified (GeneJET PCR purifications kit, Thermo Fisher Scientific) and sequenced by a commercial DNA sequencing service (LGC Genomic GmbH, Berlin, Germany) using the ITS-2 primer. The obtained sequences were compared with sequences listed in the GenBank database using the BLAST program (Altschul et al. 1990).

Results

Light microscopy

Sarcocystis-like oocysts were detected in 66/116 (56.60 %) samples. Of these, 43/116 (37.07 %) samples were obtained from Eurasian sparrowhawks and 23/116 (18.97 %) samples originated from northern goshawks. These 66 samples were selected for further examinations.

Semi-nested polymerase chain reaction specific for *Sarcocystis calchasi*

S. calchasi-specific DNA was detected in 6/116 (5.17 %) microscopically positive samples (3 Eurasian sparrowhawks, 3 northern goshawks). The remaining 60 samples did not contain *S. calchasi*-specific DNA and were submitted for sequencing.

Sequencing of ITS-1 region

All in all, 60/116 (51.72 %) samples fulfilled the requirements for sequencing of the ITS-1 region. An amplicon of the same size (approximately 840 bp) as the positive control (*S. calchasi*, 838 bp) was obtained from 39/116 (33.62 %) samples by PCR and subsequently sequenced. The amount of the PCR product of 4/116 (3.36 %) samples was too small for extraction and 17/116 (14.65 %) samples showed no visible amplicon.

Genotyping

Overall, 39 samples revealed an amplicon size similar to *Sarcocystis* sp., which was confirmed by sequencing and alignment analysis in 27 cases. DNA from *Sarcocystis turdusi/S. sp. ex A. nisus* was detected in 18/116 (15.52 %) samples originating from 16 Eurasian sparrowhawks and 2 northern goshawks. *S. columbae* DNA was detected in 3/116 (2.59 %) samples (2 northern goshawks, 1 sparrowhawk). Further, *S. cornixi* DNA was detected in 3/116 (2.59 %) samples (2 northern goshawks, 1 sparrowhawk). In 3/116 (2.59 %) samples from sparrowhawks DNA from *S. sp. ex P. carbo* was detected. In 12 cases, the sequences were either too short (2/116) for sequence comparison, belonged to ITS-regions of the *Toxocaridae* family (3/11), *Onchocercidae* family (1/116) or could not be compared to any known sequence (6/116). Results of the molecular investigation of *Sarcocystis* in sparrowhawks and northern goshawks are presented in Table 1.

Table 1 Results of *Sarcocystis calchasi*-specific real-time PCR and sequencing of ITS-1 region of samples that contained *Sarcocystis*-like oocysts

Name	Sample	Semi-nested polymerase chain reaction specific for *S. calchasi*	ITS-PCR performed	Amplicon approximal 840 bp	Sent for Sequencing	Sequence	Type locality, federal state Germany
LM 1178	2	−	+	+	+	*S. columbae*	NG
LM 1186	2	−	+	+	+	*S. columbae*	Munich, Bavaria
LM 1232	3	−	+	+	+	*S. columbae*	Illertissen, Bavaria
LM 1254	1	−	+	+	+	*S. cornixi*	Mössingen, Baden-Württemberg
LM 1251	4	−	+	+	+	*S. cornixi*	Steinhaus/Fulda Hesse
LM 1265	4	−	+	+	+	*S. cornixi*	Giesel/FD, Hesse
LM 1182	1	−	+	+	+	*S. t/S.* sp. ex *A. nisus*	Uelzen, Lower Saxony
LM 1183	1	−	+	+	+	*S. t/S.* sp. ex *A. nisus*	Giessen, Hesse
LM 1187	1	−	+	+	+	*S. t/S.* sp. ex *A. nisus*	Mössingen, Baden-Württemberg
LM 1189	1	−	+	+	+	*S. t/S.* sp. ex *A. nisus*	Berlin, Berlin
LM 1191	1	−	+	+	+	*S. t/S.* sp. ex *A. nisus*	Berlin, Berlin
LM 1195	1	−	+	+	+	*S. t/S.* sp. ex *A. nisus*	Mössingen, Baden-Württemberg
LM 1198	1	−	+	+	+	*S. t/S.* sp. ex *A. nisus*	Böblingen, Baden-Württemberg
LM 1204	1	−	+	+	+	*S. t/S.* sp. ex *A. nisus*	Milders FD, Hesse
LM 1246	1	−	+	+	+	*S. t/S.* sp. ex *A. nisus*	Giessen, Hesse
LM 1247	1	−	+	+	+	*S. t/S.* sp. ex *A. nisus*	Giessen, Hesse
LM 1271	1	−	+	+	+	*S. t/S.* sp. ex *A. nisus*	Steinsee Niederseen, Bavaria
LM 1238	3	−	+	+	+	*S. t/S.* sp. ex *A. nisus*	Trais-Lunda, Hesse
LM 1239	3	−	+	+	+	*S. t/S.* sp. ex *A. nisus*	Schlitz/Vogelsberg, Hesse
LM 1240	3	−	+	+	+	*S. t/S.* sp. ex *A. nisus*	NG, Hesse
LM 1244	3	−	+	+	+	*S. t/S.* sp. ex *A. nisus*	Wetter Kr. MR, Hesse
LM 1250	3	−	+	+	+	*S. t/S.* sp. ex *A. nisus*	Niddatal, Hesse
LM 1233	4	−	+	+	+	*S. t/S.* sp. ex *A. nisus*	Giesel/FD, Hesse
LM 1236	4	−	+	+	+	*S. t/S.* sp. ex *A. nisus.*	Blankenau/FD, Hesse
LM 1188	1	−	+	+	+	*S.* sp. ex *Phalacrocorax carbo*	Berlin, Berlin
LM 1205	1	−	+	+	+	*S.* sp. ex *Phalacrocorax carbo*	Tann, Fulda, Hesse
LM 1259	1	−	+	+	+	*S.* sp. ex *Phalacrocorax carbo*	NG, Lower Saxony
LM 1200	1	+	−	−	−	*S. calchasi*	Stuttgart, Baden-Württemberg

Table 1 (continued)

Name	Sample	Semi-nested polymerase chain reaction specific for *S. calchasi*	ITS-PCR performed	Amplicon approximal 840 bp	Sent for Sequencing	Sequence	Type locality, federal state Germany
LM 1202	1	+	−	−	−	*S. calchasi*	Giessen, Hesse
LM 1270	1	+	−	−	−	*S. calchasi*	Mönchengladbach, North Rhine-Westphalia
LM 1213	2	+	−	−	−	*S. calchasi*	Wetter MR, Hesse
LM 1252	2	+	−	−	−	*S. calchasi*	Düsseldorf, North Rhine-Westphalia
LM 1266	4	+	−	−	−	*S. calchasi*	Petersberg, Hesse
LM 1185	1	−	+	−	−	–	Mössingen, Baden-Württemberg
LM 1196	1	−	+	(+)	−	–	Vogelsberg/Monnrock, Hesse
LM 1201	1	−	+	−	−	–	Hof, Bavaria
LM 1228	1	−	+	−	−	–	Regenstauf, Bavaria
LM 1229	1	−	+	(+)	−	–	Salzgitter, Lower Saxony
LM 1230	1	−	+	−	−	–	Uetze, Lower Saxony
LM 1260	1	−	+	−	−	–	Norddeich, Lower Saxony
LM 1442	1	−	+	−	−	–	Butzbach, Hesse
LM 1177	2	−	+	−	−	–	Melsbach, Rhineland-Palatinate
LM 66	2	−	+	−	−	–	Wolfsburg, Lower Saxony
LM 1262	4	−	+	(+)	−	–	Hobieber, Hesse
LM 1277	5	−	+	−	−	–	Borsingshausen, Lower Saxony
LM 1278	5	−	+	−	−	–	Porta Westphalica, North Rhine-Westphalia
LM 1280	5	−	+	−	−	–	Giessen, Hesse
LM 1283	5	−	+	−	−	–	Osteel, Lower Saxony
LM 1274	6	−	+	−	−	–	Mönchengladbach, North Rhine-Westphalia
LM 1275	6	−	+	−	−	–	Mönchengladbach, North Rhine-Westphalia
LM 1276	6	−	+	−	−	–	Mönchengladbach, North Rhine-Westphalia
LM 1282	6	−	+	(+)	−	–	Mönchengladbach, North Rhine-Westphalia
LM 1287	6	−	+	−	−	–	Giessen, Hesse
LM 1214-1	2	−	+	+	+	*S. t/S.* sp. ex *A. nisus*[a]	Steinhaus/Fulda, Hesse
LM 1263	4	−	+	+	+	*S. cornixi*[a]	Steinhaus/FD, Hesse
LM 1181	1	−	+	+	+	*Toxocara cati*	Horas/FD, Hesse
LM 1197	1	−	+	+	+	No result	Weimar, Thuringia
LM 1214-2	2	−	+	+	+	*Porrocaecum sp.*	Steinhaus/Fulda, Hesse
LM 1261	2	−	+	+	+	*Porrocaecum sp.*	Hanover, Lower Saxony
LM 1231	1	−	+	+	+	*Onchocerca sp.*	Wetterau, Hesse
LM 1184	1	−	+	+	+	No result	Parsdorf, Bavaria
LM 1206	1	−	+	+	+	No result	NG
LM 1209	1	−	+	−	−	–	Götzenhof, Hesse
LM 1248	1	−	+	+	+	No result	Kirchdaum, Rhineland-Palatinate

Table 1 (continued)

Name	Sample	Semi-nested polymerase chain reaction specific for *S. calchasi*	ITS-PCR performed	Amplicon approximal 840 bp	Sent for Sequencing	Sequence	Type locality, federal state Germany
LM 1180	2	−	+	+	+	No result	Steinwand/FD, Hesse
LM 1255	2	−	+	+	+	No result	Giessen, Hesse

1, Sparrowhawk (intestine collected in necropsy); 2, northern goshawk (intestine collected in necropsy); 3, sparrowhawk (organ samples); 4, northern goshawk (organ samples); 5, sparrowhawk (faecal sample); 6, northern goshawk (faecal sample); +, positive; −, negative; (+), weak Amplicon; *S. t/S.* sp. ex *A. nisus*, *Sarcocystis turdusi/S.* sp. ex. *Accipiter nisus*

NG not given

[a] Sequence is too short for scientific statement

Discussion

European *Accipiter* hawks are known definitive hosts of several *Sarcocystis spp.* with avian intermediate hosts (Mehlhorn and Heydorn 1978; Olias et al. 2011; Svobodova 1996). Since oocysts of different *Sarcocystis* species cannot be distinguished microscopically, sequencing of species-specific DNA fragments is an effective way to distinguish these apicomplexan parasites. In the present study, the ITS-1 region of samples containing *Sarcocystis*-like oocysts were sequenced and compared to published sequences of *Sarcocystis* species. Although 66/116 (56.04 %) samples contained *Sarcocystis*-like oocysts, only 33 could be identified either *S. calchasi*-specific PCR 6/116 (5.17 %) or sequencing of the ITS-1 region 27/116 (23.28 %). Besides *S. columbae* and *S. calchasi*, which are known to infect *Accipitridae* (Olias et al. 2011), three *Sarcocystis* species have been found, which had not been described in *Accipiter* hawks previously.

The majority of samples, 18/116 (15.52 %), submitted for sequencing contained oocysts of *S. turdusi/S.* sp. ex *A. nisus.* Blackbirds have been previously described as intermediate hosts for *S. turdusi* (Kutkiene et al. 2012). *Accipiter* hawks and common blackbirds are in a prey-predator relationship, thus closing the life cycle of *S. turdusi* with *Accipiter* hawks as definitive hosts. The higher occurrence of *S. turdusi* in sparrowhawks (16 positive) compared to northern goshawks (2 positive) may be based on the larger number of sparrowhawks included in the study (85/116, 73.28 %). Alternatively, common blackbirds represent a larger part of the prey spectrum of sparrowhawks compared to that of northern goshawks (Kramer 1972; Fischer 1995), therefore making it more likely of *S. turdusi* being transferred to sparrowhawks rather than to northern goshawks. A solid differentiation between *S. turdusi* and *Sarcocystis* sp. ex *A. nisus* is not possible using the currently published sequences of these species (Kutkiene et al. 2012). The sequences determined in the study aligned with significant homology to both species. These results are shown in Table 2.

Sarcocystis sp. ex *Phalacrocorax carbo* was detected in three samples of sparrowhawks, even though cormorants are not a typical prey species (Kramer 1972). This suggests that cormorants may not be the only intermediate host of *S.* sp. ex *Phalacrocorax carbo.* It is not uncommon for *Sarcocystis* species to have several intermediate hosts (Tadros and Laarman 1982). Further investigations may detect more intermediate hosts for this species.

S. cornixi was found in two samples of northern goshawks and one sample of a sparrowhawk. In correlation to these findings, *Corvidae* species are part of the prey spectrum of northern goshawks and sparrowhawks (Kramer 1972; Fischer 1995), thus fulfilling the life cycle of *S. cornixi* with high certainty.

S. columbae is a known parasite of *Accipiter* hawks (Olias et al. 2011). In this study, three samples contained S. *columbae* DNA. This seems to state a far lower infection rate than published previously (84 % of northern goshawks, 85 % of Eurasian sparrowhawks) (Olias et al. 2011). The sample collection of the present study was spread throughout Germany, which includes various habitats of *Accipiter* hawks with smaller *Columbae* sp. populations. A habitat and prey-depending endemic cluster of *Sarcocystis* species could explain the seemingly controversial low number of *S. columbae*-infected *Accipiter* hawks in the present study. Only samples which contained microscopically detectable oocysts where included in this study. Thus may had also altered the detection rate. This disadvantage was acceptable because the goal of the study was not to estimate the prevalence of known *Sarcocystis* sp. but to detect new *Sarocystis* species in *Accipiter* hawks.

DNA of *S. calchasi* was detected by the *S. calchasi*-specific real-time PCR in six cases. This PCR is highly specific for *S. calchasi*; therefore, further sequencing of those samples was not performed (Maier et al. 2014). *Accipiter* hawks are known definitive hosts of *S. calchasi*, *Columbiformes* and *Psittaciformes* are already identified as intermediate hosts.

Table 2 Variable sites within the ITS-1 region

Species	GenBank accession number	Site number						
		292	296	314	463	730	758	790–795
Sarcocystis sp. ex *Accipiter nisus*	GU253886	C	C	G	T	T	T	TAA–G
S. turdusi Eru 1	KJ540167	T	C	A	T	C	T	TGAATG
S. turdusi M 41	JF975683	T	C	A	A	C	A	TGAATG
S. turdusi M 42	JF975684	C	A	A	T	C	A	TGAATG
S. turdusi M 51	JF975685	T	N	A	T	C	A	TGAATG
S. turdusi Tph 12	KJ540166	T	C	A	T	T	T	TGAATG
S. turdusi Tpi 1	KJ540164	T	C	A	T	C	A	TGAATG
S. turdusi Tpi 2	KJ540165	T	A	A	T	C	T	TGAATG
S. turdusi Tph 15	KT588510	T	C	A	T	C	T	GAATGA
LM 1182		T	C	A	T	C	T	TGAA
LM 1183		C	C	A	T	T	A	TGAA
LM 1187		C	A	A	T	T	A	–
LM 1189		C	A	A	T	T	A	–
LM 1191		T	C	A	T	–	–	–
LM 1192		T	C	A	T	T	T	TGAA
LM 1195		C	A	A	T	T	A	TGAA
LM 1198		C	A	A	T	T	–	–
LM 1204		C	C	A	T	T	–	–
LM 1205		C	C	–	–	–	–	–
LM 1233		–	–	–	T	–	–	–
LM 1236		C	A	A	A	T	T	TGAA
LM 1238		C	C	A	T	C	T	–
LM 1239		–	–	–	–	T	T	TGAATG
LM 1244		T	C	A	T	T	–	–
LM 1246		T	A	A	T	C	T	TGAATG
LM 1247		T	A	A	T	T	T	TGAATG
LM 1250		C	C	A	T	T	T	–
LM 1271		T	C	A	T	C	T	–
LM 1400		C	A	A	A	T	T	–

Multiple infections with two or more *Sarcocystis* species could not be detected with the method used in the present study, and their interference with an effective sequencing may be an explanation for low yields of significant results. If the infection with one *Sarcocystis* species has dominated an infection with another *Sarcocystis* sp., the amplification of the ITS-1 region of the less abundant species may be inhibited. Since parallel infections with several *Sarcocystis* species seem to be a frequent event in *Accipiter* hawks (Olias et al. 2011), the relatively high number of inconclusive results is explicable. Additionally, parallel infections with other parasites than *Sarcocystis* spp. were found frequently by microscopical examination, which may explain the finding of DNA from *Toxocaridae* and *Onchocercidae*. The interference of these parasites with the amplification of *Sarcocystis* DNA was not investigated in our study, however seems to be likely.

The 116 samples investigated in this study do not represent an epidemiological collection. The aim of this study was to determine which *Sarcocystis* species are present in samples of *Accipiter* hawks collected throughout Germany. The higher infection rate of *S. turdusi/S.* sp. ex *A. nisus* compared to those of other detected *Sarcocystis* species is a rough estimation, and further investigation on the prevalence should follow. However, *S. turdusi*, *S. cornixi* and *Sarcocystis* sp. ex *Phalacrocorax carbo* have been identified in their natural definitive hosts for the first time and their life cycles have thus been completed.

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Compliance with ethical standards All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

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