Molecular Epidemiology of the Neglected Meat-Borne Pathogen *Sarcocystis* Spp. in Pigs and Cattle: an Update

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Abstract

The host specific *Sarcocystis* spp., some of them with zoonotic character, is considered cyst-forming intracellular coccidian (Apicomplexa: Sporozoa) parasites. Their life cycle involves carnivores as definitive hosts and herbivores and omnivores as intermediate hosts, including pigs and cattle. The present work aimed to review the worldwide distribution and molecular epidemiology of *Sarcocystis* spp. in cattle and pigs, processing PubMed retrieved relevant scientific papers, published in the new century and based on molecular tools. Mainly, prevalence values of the infections according to countries and geographical regions; incriminated *Sarcocystis* spp. and their isolation sources together with the targeted genes and used primers, as well as public health significance of the isolates, are presented. The published summarized results may be useful for epidemiologists and public health specialists**.**

Keywords: epidemiology, molecular, review, *Sarcocystis.*

1. General considerations

Sarcocystis species are recognized as two host cycle, host specific, coccidian parasites infecting a wide range of warm-blooded and poikilothermic animals. Humans, as final host beside other carnivores (canids, felids), become infected through consumption of raw or undercooked pork or beef containing infecting stages (mature sarcocysts with bradyzoites) of the zoonotic *Sarcocystis* spp [1, 2]. The most common consequences of the illness in humans, namely sarcocystosis, include appetite loss, vomiting, nausea, diarrhea, abdominal pain, respiratory disorders or tachycardia [3].

Currently, based on molecular investigations cattle are known harboring five *Sarcocystis* species, namely *S. cruzi*, *S. hirsute*, *S. hominis*, *S. rommeli* and *S. heydorni* [2]. In addition, the frequently recorded *S. sinensis* with an unknown life cycle, even if it is reported in many publications, currently is considered a *nomen nudum* [1].

Nowadays, the presence of other two genetically distinct species, *S. bovifelis and* S. *bovini,* has been recorded [4]. Out of them, only *S. hominis* and *S. heydorni* are considered to have zoonotic character. Domestic and feral pigs (wild boars) are intermediate hosts for three species including *S. miescheriana*, *S. suihominis,* and *S. porcifelis,* but only *S. suihominis* is zoonotic [2]. In veterinary clinical practice, the routine meat inspection of the slaughtered animals under light microscopy (i.e. pork inspection for *Trichinella* detection) is not enabling to differentiate the detected infecting stages of the parasite at the species level, leaving public health authorities with data indicating the presence of *Sarcocystis*, but no additional important information of the findings with public health significance. To cover this shortfall, the molecular biological methods (conventional PCR, sequencing) are considered reliable tools.

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2. Study design

The data for the current update covers the period from 2009 to nowadays. The processed relevant scientific papers were obtained after PubMed searching using *Sarcocystis* and sarcocystosis terms combined with pig, swine, wild boar and pork, or cattle, bovine and beef, respectively. Therefore, a total of 18 representative papers were selected, which in author's opinion represents more than 60% out from the available published information from the scientific literature in the last 6 years.

3. Epidemiological aspects

The results of the current update they are synthetically presented in the Table 1.

Table 1. Epidemiological data on the occurrence of *Sarcocystis* spp. in different countries at worldwide level in pigs, wild boar and cattle based on molecular investigations

Host	*County (geographical region)*	*No. of Sarcocystis positive samples / examined (%prevalence)*	*Isolation source (tissue)*	*Targeted gene region/primer set*	*Identified species (%)/no. of sequenced isolates*	*References*
Sus scrofa domesticus	Switzerland	1* – case study	myocardium	18S rRNA / COC1 and COC2	*S. miescheriana*	Caspari et al. 2011
	China (Henan Province-Central region)	4/4 (100%) – isolates for slaughtered pigs	diaphragm	18S rRNA / S18SF and S18SR	*S. miescheriana*/4	Yan et al. 2013
	India (Punjab)	182/250 (72.8%)	myocardium	18S rRNA /	*S. miescheriana* (n=1), *S. suihominis* (n=6)/7	Kaur et al. 2016
Sus scrofa	Portugal (North-Eastern)	76/103 (73.8)	diaphragm	18S rRNA / Sar-F, Sar-R, SmiesF and SsuihR	*S. miescheriana*/19	Coelho et al. 2015
	United States of America	44/147 (29.9)	myocardium	18S rRNA / 2L and 3H	*S. miescheriana*/31	Calero - Bernal et al. 2015
	Spain	8/25 (32)	myocardium	18S rRNA / 2L , 2H and 3H	*S. miescheriana* (n=7), *S. suihominis* (n=1)/8	Calero - Bernal et al. 2015
	Iran	1*	thigh muscle	18S rRNA / SarcoF and SarcoR	*S. miescheriana*	Kia et al. 2011
Bos taurus	Hungary	100/151 (66)	oesophagus myocardium	18S rRNA / COC1 and COC2; BJ1 and BN2	*S. cruzi* (64), *S. hominis* (19), *S. sinensis* (17)/36	Hornok et al. 2015
	Germany	174/257 (67.7)	minced (muscle) beef	18S ribosomal DNA/ SarcoFext and SarcoRext	*S. sinensis* (n=22), *S. hominis* (n=5) and S. *hirsuta* (n=1)/28	Moré et al. 2014
	Argentina	135/380 (35.5)	loin	18S rRNA / SarcoF and SarcoR	*S. cruzi* (n=16), *S. hominis* (n=13)	Moré et al. 2011
	Italy (North-Western)	279/384 (72.7) 228/384 (59.4)	oesophagus diaphragm	18S rRNA / -	*S. cruzi* (285*), S. hominis*	Domenis et al. 2011

Host	*County (geographical region)*	*No. of Sarcocystis positive samples / examined (%prevalence)*	*Isolation source (tissue)*	*Targeted gene region/primer set*	*Identified species (%)/no. of sequenced isolates*	*References*
		225/384 (58.6)	myocardium		(164), *S. hominis*-like (71), *S. hirsuta* (7)	
	Iran	90/101 (89.1)	diaphragm	18S rRNA / designed primers	*S. cruzi* (89), *S. hominis* (48)	Akhlaghi et al. 2016
		40/123 (32.5)	oesophagus diaphragm myocardium intercostals muscle	18S rRNA / 18S1H and 18S9L	*S. cruzi*	Hamidineja t et al. 2015
		1*	hamburger	18S rRNA / SarF and SarR	*S. hominis*	Ahmadi et al. 2015
			diaphragm	18S rRNA	*S. hominis*	Hajimoham madi et al. 2014
	Malaysia	49/77 (63.6)	myocardium	18S rRNA	*S. cruzi* (49)	Latif et al. 2015
	Vietnam	63/101	oesophagus, diaphragm, tongue and cervical muscle	18S rRNA / 18S9L and 18S1H	*S. cruzi* (55*), S. hominis* (54), *S. hirsuta* (28)	Jehle et al. 2009
	Argentina	147 excised individuals sarcocysts from 12 samples of cattle meat	beef muscle tissue	Partial *Cox*1/SF1 and SR9 and for 7 isolates of *S. bovifelis*	*S. bovifelis* (n=67), S. *bovini* (5), *S. hirsuta* (1), *S. cruzi* (n=21)	Gjerde, 2016
	New Zeeland			SR8D and and SR9; and Ss1R, Ss2R for *S. bovifelis*, Sb1R and Sb2R for S. bovini	*S. bovifelis* (n=38), S. *bovini* (19), *S. hirsuta* (19)	
	Brazil				*S. bovifelis* (n=15), *S. hirsuta* (15)	
	Uruguay				*S. bovifelis* (n=1), *S.cruzi* (1)	
	Germany				*S. bovifelis* (n=26), S. hirsuta (n=21)	

Legend: * - percentage not shown because of low sample number;

Domestic pigs (*Sus scrofa domesticus*)

The first report of naturally acquired clinical sarcocystosis in a domestic pig caused by *S. miescheriana* has been reported as a case study by Caspari et al. [5] in a pig breeding stock in Switzerland. The infection has been molecularly confirmed targeting the conserved region of the small-subunit rRNA gene and using COC1 and COC2 specific primer set. The protozoa isolation source was the cardiac tissue [5].

In China, four *Sarcocystis* isolates from slaughtered pigs have been molecularly characterized by Yan et al. [3] in Henan Province, central region. PCR amplification of 18S rRNA

gene using S18SF and S18SR primers demonstrates the occurrence of *S. miescheriana* [3]. In contrast, the dominance of the zoonotic *Sarcocystis* infection due to *S. hominis* has been recently reported in India, Punjab region. The study results highlighted the contribution of the pig rearing under unhygienic conditions near human settlements at the occurrence of zoonotic sarcocystosis in domestic pigs in this geographical area [6].

Wild boar (***Sus scrofa***)

The widespread occurrence of the non-zoonotic *S. miescheriana* circulating in wild boars in northeastern of Portugal has been recently demonstrated by Coelho et al. [7]. Thus, after processing diaphragm tissue samples a 73.8% infection rate (76/103) has been obtained using *Sarcocystis* genus – specific PCR and Sar-F and Sar-R primer set. Subsequently, *S. miescheriana* specific PCR showed positive results for all these samples, as well as sequencing in 19 situations. In addition, mature adults (≥26 months old) and young adults (between 15 and 25 months old) age categories, as well as female wild boars were identified as risk factors for *Sarcocystis* spp. infection [7].

In a study conducted by Calero-Bernal et al. [8] in 29 states of the United States of America, the *Sarcocystis* 18S rRNA gene has been successfully amplified in 44 out from 147 examined heart samples using the 2L and 3H primer set. RFLP analysis of the amplicons showed the presence of only one species, namely *S. miescheriana*, which have been confirmed for 31 samples through sequencing [8].

The occurrence of *S. miescheriana* in a hunted wild boar in the temperate area of northern Iran has been reported by Kia et al. [9]. The pathogen was isolated from the thigh muscle tissue and molecularly confirmed amplifying a fragment of *Sarcocystis* 18S rRNA gene using the Sarco-F and Sarco-R primers and sequencing [9]. Recently, the identity of the zoonotic *S. suihominis* (1/8) in the wild boar meat intended for human consumption, as first record on this host from Europe, has been confirmed through PCR-RFLP procedure and sequencing in Southwestern areas of Spain. Other registered species in this study was the widespread *S. miescheriana* (7/8) [10].

Cattle (*Bos taurus*)

In a countrywide survey carried out in Hungary by Hornok et al. [11], a total of 151 beef cattle providing from 31 places have been molecularly investigated for the presence of *Sarcocystis* spp., processing oesophagus and heart muscle samples. The results showed the presence of three genetically distinct species namely, *S. cruzi* (64%), *S. hominis* (19%) and *S. sinensis* (17%), respectively [11].

In a German study carried out by Moré et al. [12], a total of 257 beef samples obtained from different butcheries and supermarkets were investigated for *Sarcocystis* spp. Out of them 174 (67.7%) were found to be *Sarcocystis* positive after processing with conventional PCR and 179 (69.6%) by multiplex real-time PCR, respectively. Sequencing of 28 selected samples showed the dominance of *S. sinensis* (n=22) and the presence of other two species, namely *S. hominis* (n=5) and S. *hirsuta* (n=1) [12].

The prevalence of *Sarcocystis* spp. in Argentinean cattle was evaluated by Moré et al. [13], processing molecularly a total of 380 loin samples. Amplification products, using SarcoF and SarcoR primers, were observed in 35.5% (135/380) of the analyzed samples. RFLP analysis demonstrated restriction patterns corresponding with *S. cruzi* (n=16) and *S. hominis* (n=13) [13].

A high genetic diversity of *Sarcocystis* isolates has been recorded in northwestern Italy in a prevalence study of sarcosporidiosis in semi-intensively bred cattle. From a total of 384 slaughtered cattle esophagus, diaphragm and heart samples were collected and processed for *Sarcocystis* detection. Molecular characterization of the isolates targeting the 18S rRNA gene showing the prevalence of *Sarcocystis* spp. in the sampled population under different types of associations. The overall prevalence of the registered species was as follows: *S. cruzi* 74.2% (n=285), *S. hominis* 42.7% (n=164), *S. hominis*-like 18.5% (n=71), and *S. hirsuta* 1.8% (n=7), respectively. Out from the tested matrices the esophagus showed the highest number (n=9) of different species associations, followed by the diaphragm (n=6) and the heart (n=4) samples. Also, the most often parasitized anatomic structure was the esophagus (n=279), followed by the diaphragm (n=228) and the heart (n=225) [14]

The dominance of *S. cruzi* beside the zoonotic *S. hominis* has been recently reported in a new

primer and restriction enzyme designed study in Iran. The presence of pathogen has been molecularly demonstrated in 98.9% (90/91) of microscopically positive samples meaning diaphragmatic muscle from slaughtered cattle. The results of species determination showed the presence of *S. cruzi* (89/90) and *S. hominis* (48/90) under different combination forms, but the lack of *S. hirsuta* [15]. In another study, conducted in the same country ,only the presence of *S. cruzi* has been molecularly confirmed in 40 microcyst positive slaughtered cattle samples, having different isolation sources (oesophagus, diaphragm, myocardium, and intercostals muscle) [16]. In addition, the presence of *S. hominis* in a 6-year-old native cow slaughtered at Yazd Province [17] as well as in a traditional hamburger intended to human consumption [18] has been reported.

Sarcocystis spp. distribution in different anatomical structures of cattle (oesophagus, diaphragm, tongue and cervical muscle) has been PCR-RFLP studied in Vietnam. Samples providing from 63 microscopically *Sarcocystis* positive cattle harbored different species under different combination forms as follows: *S. cruzi*+*S. hominis*+*S. hirsuta* (n=23), S. *cruzi*+*S. hominis* (n=23), S. *cruzi*+S. *hirsuta* (n=2), *S. hominis*+*S. hirsuta* (n=3), *S. cruzi* (n=7), and *S. hominis* (n=5), respectively [19].

In the most recently published report, Gjerde et al. [4] molecularly analyzed 147 excised individuals sarcocysts from 12 samples of cattle meat providing form Argentina, New Zeeland, Brazil, Uruguay and Germany. The identified species were *S. cruzi*, *S. hirsuta*, *S. bovifelis* and *S. bovini*. The last two species was newly recognized genetically distinct parasites and were reported for the first time in cattle according to the presented data in the Table 1.

4. Conclusions

The summary of the available scientific papers dedicated to the study of the molecular epidemiology of *Sarcocystis* infections in pigs and cattle demonstrated a scanty background, especially in European countries, even if the food safety regulations require the surveillance of protozoa in meat and derivate products by each member state.

The published results about the distribution of *Sarcocystis* spp. in domestic pigs and wild boar showed a widespread occurrence of *S. miescheriana*, a narrower appearance of *S. suihominis* (only 2 studies) and the lack of *S. porcifelis* with uncertain taxonomic status as previously described [2].

In bovine *Sarcocystis* infections *S. cruzi* and the zoonotic *S. hominis* seems to be the most prevalent species, followed by *S. sinensis* (currently recognized as nomen nudum) and *S. hirsuta*. In addition, the presence of a considerable number of *S. bovifelis* and *S. bovini* has been reported in a single study, and currently they can be considered genetically distinct species [4].

The relatively reduced number of publications presenting *Sarcocystis* molecular epidemiologic background in livestock meat intended for human consumption, pointed out the necessity of the increasing the number of surveys dedicated to this topic at worldwide level and special emphasis on European countries. Also, the increasing of the adequately trained scientists in this field or the improvement of training the meat control personnel can significantly contribute to the better knowledge of the molecular epidemiology of this neglected meat-borne pathogen.

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