Prevalence and Intensity of Sarcocystis Infections in Alpine Chamois (Rupicapra r. rupicapra) in Germany

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Article

Prevalence and Intensity of *Sarcocystis* Infections in Alpine Chamois (*Rupicapra r. rupicapra*) in Germany

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Abstract: Chamois are mountain ungulates (Subfamily Caprinae) which inhabit several medium and high altitude mountain ranges from southern Europe to the Near East. The first findings of *Sarcocystis* cysts in the musculature of chamois were reported in the 1970s. However, only limited work on the epidemiology of sarcocystosis and identification of the species of *Sarcocystis* in chamois has been done in the past. The present work was part of a comprehensive study on the parasites of chamois in Germany aiming to provide, for the first time, data on the prevalence and intensity of *Sarcocystis* infection in native Alpine chamois using histology examination of heart and/or diaphragm tissue samples collected from 216 chamois (40 kids and 176 chamois ranging of one up to 18 years of age). Sarcocysts were detected in either heart or diaphragm of 167/216 chamois (77.3%) with 131 of 183 heart samples and 127 of 215 diaphragm samples testing sarcocyst positive. Of the 181 chamois with both heart and diaphragm available (34 kids and animals up to 18 years of age), sarcocysts were detected in heart and/or diaphragm of 142 animals translating in an overall 78.5% prevalence of *Sarcocystis* infection (95%CI 72.5% - 84.4%). Sarcocysts were more frequently recorded in heart vs. diaphragm (72.4% vs. 56.4%; p=0.0021), and diaphragm positivity was associated with heart positivity (p=0.0001). Sarcocyst positivity (heart and/or diaphragm) was significantly (p<0.001) lower in kids than in the older chamois (27.1% vs. 88.6%, respectively) but prevalence did not differ between the sexes, regardless of the chamois' age (p>0.3). Intensity of infection was generally low (<10 sarcocysts per cm² muscle cut) in both heart positive and diaphragm positive animals (94.7% and 93.7%, respectively) with heart yielding higher sarcocyst counts than diaphragm (p<0.001). Both heart and diaphragm sarcocyst counts were significantly (p<0.001) lower in kids than in the older chamois. *Sarcocystis* infection was demonstrated to be prevalent in chamois in Germany but intensity is apparently low. Further studies are desired to identify the species of *Sarcocystis* parasitizing the chamois using both phenotypic and molecular characteristics.

Keywords: *Sarcocystis*; chamois; heart; diaphragm; Germany

1. Introduction

Chamois are mountain ungulates (Genus *Rupicaprinus*, Subfamily Caprinae, Family Bovidae) which inhabit numerous medium and high altitude mountain ranges from southern Europe to the Near East. The taxonomy of the chamois has been revised several times since the end of the 19th century. Currently, there is consensus to recognize two species of chamois, the Northern chamois, *Rupicapra rupicapra* (with seven subspecies, occurring from central-eastern Europe to the Near East) and the Southern chamois, *Rupicapra pyrenaica* (with three subspecies, occurring in south-western Europe). The Alpine chamois, *R. r. rupicapra* is the most abundant subspecies of the two species of chamois. It is native to the whole Alpine Mountain range and has been also successfully introduced into former Czechoslovakia (parts of both todays Czech Republic and Slovakia) and into New Zealand where it forms a significant population on the South Island. However, several subspecies of *R. rupicapra* and *R. pyrenaica* require conservation measures [1].

With respect to the intracellular cyst forming protozoan parasites of the genus *Sarcocystis*, review of the literature has shown that findings of sarcocysts (*Sarcocystis* intramuscular stages) in chamois were apparently for the first time reported at almost the same time in Alpine chamois (*R. r. rupicapra*) from Switzerland [2,3], former Czechoslovakia [4,5] and Italy [6]. Subsequent work, mainly based on

conventional histology, reported further findings of sarcocysts in Alpine chamois from France [7-9], Italy [10-15], Switzerland [15], Austria [16,17], Germany [16,19], former Czechoslovakia/Bohemia [20] and Slovakia [21]; in Tatra chamois (*R. r. tatrica*) from Poland [22]; in Carpathian chamois (*R. r. carpatica*) from Romania [23]; in Cantabrian chamois (*R. p. parva*) from Spain [24-26], and in Pyrenean chamois (*R. p. pyrenaica*) from Spain [27,28].

However, only limited work regarding an adequate identification of the species of *Sarcocystis* parasitizing chamois using ultrastructural and molecular methods has been done in the past. Two species were identified and named: *S. cornagliai* described on fine structural studies from Alpine chamois from Austria, Germany and Italy [10,16] and *S. tenella* from Tatra chamois from Poland using both microscopical and molecular characteristics [22]. Sarcocysts which were morphologically similar to the *S. tenella*/*S. capracanis*-type in sheep and goats were previously described from Alpine chamois from Italy [10] and from Austria and Germany [16]. The sarcocysts of *S. cornagliai* are ultrastructurally very similar to one sarcocyst type reported from the Rocky Mountain goat (*Oreamnos americanus*) [16,29] and were recorded too in Alpine ibex (*Capra ibex*) [30]. Interestingly, a brief conference abstract from 1980 stated that sheep, goat and chamois are each host of three sarcocyst forms which are microscopically indistinguishable [19]. It is further worth to mention that there are short descriptions of two transmission experiments where sarcocysts-containing muscle tissues of chamois were fed to dogs and the sporocysts shed by the dogs developed into sarcocysts of the *S. tenella*/*S. capracanis* type in both sheep and goats [19, 31].

In addition to the scientific interest to identify and characterize the species of *Sarcocystis* parasitizing the (sub)species of chamois, which is also important for the understanding of the phylogenetic relationship of the species of intermediate hosts belonging to the Caprinae subfamily [32], several aspects on the epidemiology of these muscle parasites are still not well known in chamois and other wild ungulates which constitute, at least regionally, an important source of meat for human consumption [12,33]. Therefore, this paper presents investigations on the prevalence, distribution and intensity of *Sarcocystis* infection in Alpine chamois from Germany where the chamois occur as native species in the German part of the Alps (Bavarian Alps) and, after successful transfer in the 1930s of chamois from the Alps, in the Black Forest mountains [34].

2. Material and methods

In the conjunction with studies on the endoparasite fauna of Alpine chamois in Germany [35, 36] heart and/or diaphragm muscle samples of 216 chamois (40 kids and 176 chamois ranging of one up to 18 years of age) were collected for examination for sarcocysts (*Sarcocystis* spp. infection). The samples were collected from chamois harvested according to the hunting regulations during the years of 2004 to 2006 (179 chamois originated from the Bavarian Alps, ranging from the National Park Berchtesgaden in the east to the Allgäu Alps in the west, and 37 chamois from the Black Forest mountains); information on age and sex of the animals was provided by the hunters. Both heart and diaphragm tissues were available for examination from 181 chamois; only heart or diaphragm muscle were available from two or 34 chamois, respectively.

For analysis, animals were stratified in four classes considering sexual maturity and contribution to the reproduction of the population in addition to age [37]: Kids (<1 year old); Class 3, junior class (1 to 2 years old males, 1 to 3 years old females); Class 2, middle class (3 to 7 years old males, 4 to 9 years old females); Class 1, senior class (≥8 years old males, ≥10 years old females). Reproduction of the chamois population is based on the sexually mature adult middle class (primarily) and senior class chamois while kids (sexually immature) and junior class chamois, although sexually mature, do not contribute to the reproduction of the population [37]. The total sample set of 216 chamois included 40 kids (17 male, 23 female), 72 junior class chamois (37 male, 33 female, 2 unknown sex), 85 middle class chamois (63 male, 22 female) and 19 senior class chamois (9 male, 10 female). The subsample of 181 chamois from which both heart and diaphragm tissues were available (160 and 21 originating from the Bavarian Alps and Black Forest mountains, respectively) included 34 kids (16 male, 18 female), 72 junior class chamois (31 male, 25 female, 1 unknown sex), 74 middle class chamois (55 male, 19 female) and 16 senior class chamois (7 male, 9 female).

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Standard histological examination (light microscopy) was done on formalin-fixed tissue specimens (right ventricular wall, diaphragmatic pillars), using hematoxylin-eosin-stained 5 µm sections of paraffin embedded tissue. One section per tissue per animal was examined for intramuscular sarcocysts (*Sarcocystis* cysts). For each sample, the area of tissue examined was determined using the imaging software dhs-Bilddatenbank® V5.0 (Dietermann&Heuser, Greifenstein, Germany) to allow for the estimation of the intensity of infection as number of sarcocysts per cm^2 muscle cut.

To assess the relationship between sarcocyst positivity or presence (interpreted as prevalence of *Sarcocystis* species infection) and host demographic factors and for the analysis of the intensity of infection (number of sarcocysts per cm^2 muscle cut, sarcocyst count), the subsample of 181 chamois with both heart and diaphragm tissues examined was considered.

Associations between sarcocyst positivity and variables representing host demographic factors (age class, sex) were assessed using contingency tables and Fisher's exact test or Chi-square statistics. The association of sarcocyst positivity in heart and diaphragm tissues was analyzed using McNemar's test for matched-pair samples. Intensity of infection was analyzed using the Kruskal-Wallis test or the Mann-Whitney test, as applicable. All testing was two-sided, and level of significance for all analyses was set at $p<0.05$.

Spearman's rank correlation coefficient (r_s) was calculated to assess the association of the sarcocyst counts of the two tissues and the association of the sarcocyst counts with the age of the chamois.

3. Results

Overall, of the 216 chamois examined, 167 (77.3%) yielded evidence of infection with *Sarcocystis* species as determined by histological detection of sarcocysts in either heart or diaphragm specimens. There was no difference in sarcocyst positivity between the chamois from the Bavarian Alps and the Black Forest mountains (140/179, 78.2% and 27/37, 72.9%; p=0.5196).

The examination for sarcocysts of the 181 chamois with both heart and diaphragm tissues available demonstrated a collective prevalence of *Sarcocystis* infection of 78.5% (95%CI 72.5% - 84.4%). Sarcocysts were more frequently recorded in heart vs. diaphragm (131/181, 72.4% vs. 102/181, 56.4%; p=0.0021). Diaphragm sarcocyst positivity was associated with heart sarcocyst positivity (diaphragm positive [heart positive]/heart positive: 91/131, 69.5% vs. diaphragm positive [heart negative]/heart negative: 11/50, 22.0%; p=0.0001).

For the prevalence of infection in heart and/or diaphragm the Chi-square test revealed a significant ($p<0.001$) difference between kids (27.5%), junior, middle and senior class chamois (87.5%, 89.4% and 89.5%, respectively). Sarcocysts were significantly ($p<0.05$) more often recorded in the samples from junior, middle and senior class chamois than in those from kids; but there was no difference in the prevalence of *Sarcocystis* infection between the three classes of sexually mature chamois (p=0.941). No significant difference in the prevalence of *Sarcocystis* infection was found between male and female chamois of the combined four age classes (80.7% vs. 74.7%; p=0.3585) and of the combined three classes of sexually mature chamois (89.2% vs. 94.3%; p=0.3762).

Similar results were obtained considering sarcocyst positivity in heart and diaphragm samples separately: there was a significant ($p<0.001$) difference between kids (17.1% and 25.0%, respectively), junior, middle and senior class chamois (82.5% and 70.8%, 86.7% and 63.1%, and 81.3% and 68.4%, respectively) and sarcocysts were significantly ($p<0.05$) more often recorded in the samples from junior, middle and senior class chamois than in those from kids but there was no significant difference in the prevalence of *Sarcocystis* infection between junior, middle and senior class chamois ($p>0.5$). No significant difference in the prevalence of *Sarcocystis* infection was found between male and female chamois of the combined four age classes and of the combined three classes of sexually mature chamois ($p>0.5$).

Results of the examination of the samples of the 181 chamois with both heart and diaphragm tissues available for the intensity of infection are summarized in Table 1. As there was no statistical

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difference for the sarcocyst counts between the three classes of sexually mature chamois, overall and for the two sexes ($p<0.05$, Kruskal-Wallis test), data for the three classes were combined.

Table 1. Intensity-of-infection by histology (number of sarcocysts per cm² muscle cut) in the heart and diaphragm muscle tissues of 181 chamois from the Bavarian Alps and Black Forest mountains in Germany (animals with both heart and diaphragm muscle samples examined).

Age class	Sex	Number of sarcocysts per cm² muscle cut, mean ± standard deviation	
		Heart	Diaphragm
Kids (<1 year, n=34)	Male	0.26 ± 0.43	1.30 ± 1.95
	Female	0.91 ± 1.51	0.88 ± 1.47
	Male + Female	0.60 ± 1.00	1.08 ± 1.72
Combined Classes 1, 2 and 3 (actually ~1 to 18 years, n=147)	Male	3.72 ± 2.72	1.72 ± 1.63
	Female	3.44 ± 2.18	3.16 ± 2.64
	Male + Female	3.61 ± 2.51	2.26 ± 2.06
Combined kids and Classes 1, 2 and 3 (actually <1 to 18 years, n=181)	Male	3.22 ± 2.68	2.88 ± 2.36
	Female	1.66 ± 1.69	2.58 ± 2.56
	Male + Female	3.05 ± 2.55	2.04 ± 2.07

Age classes: Class 3, junior class (1 to 2 years old males, 1 to 3 years old females); Class 2, middle class (3 to 7 years old males, 4 to 9 years old females); Class 1, senior class (≥8 years old males, ≥10 years old females).

Of the 131 heart sarcocyst positive chamois, 124 and seven had <10 and ≥10 sarcocysts per cm² muscle cut, respectively. Of the 127 diaphragm sarcocyst positive chamois, 119 had <10 and eight had ≥10 sarcocysts per cm² muscle cut. Maximum intensity recorded was 18.7 sarcocysts per cm² for heart tissue and 14.6 sarcocysts per cm² for diaphragm tissue in an one and a half year old male and a two and a half year old female, respectively.

Diaphragm tissue had significantly lower counts of sarcocysts than the heart tissue of the combined four age classes and of the combined three classes of sexually mature chamois ($p<0.001$) while counts in the two tissues from the kids did not differ ($p=0.662$).

There was a significantly positive correlation of the sarcocyst counts in both heart and diaphragm tissues with the age of the chamois ($r_s=0.4038$, $p<0.001$, and $r_s=0.2093$, $p=0.005$, respectively). Kids had significantly lower counts of sarcocysts in both heart and diaphragm tissues than the older chamois ($p<0.001$).

There was no statistical difference between male and female animals for the heart sarcocyst counts of the combined four age classes, combined three classes of sexually mature chamois and kids, and for the diaphragm sarcocyst counts of the combined four age classes and of kids ($p>0.1$); however, among the combined three classes of sexually mature chamois female animals had significantly higher diaphragm sarcocyst counts than male animals ($p<0.01$).

There was a significant medium size relationship between the sarcocyst counts of the two tissues for the combined four age classes (Spearman's $r=0.4038$; $p<0.001$).

4. Discussion

The present work was part of a comprehensive study on the parasites of chamois in Germany [35,36] aiming to provide, for the first time, epidemiological data on the prevalence and intensity of *Sarcocystis* infection in chamois from Germany using histology examination of heart and/or diaphragm muscle samples collected from 216 chamois.

For the study of the prevalence and intensity of infection with *Sarcocystis* species in intermediate hosts, standard histology was used which has been shown to be suitable for epidemiological studies of *Sarcocystis* infections although enzymatic digestion was more sensitive for the detection of the

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infection as discussed previously [15,38]. Therefore, histologic examination used to determine the prevalence of infection represents a minimal estimate. Apart from the technique used for the detection of infection, the type of muscle tissue examined for sarcocysts may influence the outcome of the testing so that the results of various studies are not easily comparable [38].

While the majority of publications does not necessarily allow for conclusions as to the prevalence of *Sarcocystis* infection in chamois, mainly because of small sample size examined, lack of quantification of information or representing accidental findings [2-9,11-13,15-24,26-28], the overall prevalence of *Sarcocystis* infection established in this study with almost 80% of the animals testing sarcocyst positive is in line with the highest prevalences reported previously in the literature [10,25]. Cornaglia et al. [10] examined heart tissue of 198 Alpine chamois from the western Alps in Italy by conventional histology and recorded sarcocysts in 79.8% of the animals, Díez-Baños et al. [25] using tissue compression recorded sarcocysts in muscle tissues of 78.1% of 32 Cantabrian chamois originating from the Cantabrian range in northern Spain. A slightly lower prevalence of *Sarcocystis* infection, 65%, was reported based on the histological examination of heart and diaphragm samples of 49 Alpine chamois from the central-eastern Alps in Italy [14]. Overall, however, it seems reasonable to assume that *Sarcocystis* infections are common in chamois of both species and have been demonstrated in animals of several subspecies of chamois. In agreement with the study of Cornaglia et al. [10] in chamois and several studies in other wild ungulates in Europe including mouflon and several species of cervids [21,38-46], prevalence of sarcocyst positivity was positively correlated with the age of the animals which reflects an age- and nutrition-associated greater exposure to the infectious *Sarcocystis* stages for the older animals related with a lack of development of a robust protective immunity. Interestingly, sarcocyst positivity of chamois kids found by Cornaglia et al. [10] was more than twice the frequency recorded in the present study – ~57% vs. ~28%, respectively – while the sarcocyst positivity in the older animals was comparable.

Similarly, consistent with previous studies evaluating sex-related differences in the prevalence of *Sarcocystis* infection in wild ungulates (see 38), no difference between male and female chamois was found.

To the knowledge of the authors, only one previous study in chamois provided an estimation of the intensity of *Sarcocystis* infection, based on counts of sarcocysts in pieces of muscle tissue examined by tissue compression [25]. The present investigation generated sarcocyst counts following standard histology and it is not known to which extent histology sarcocyst counts correlate with counts established by Díez-Baños et al. [25]. According to a score proposed for the classification of sarcocyst counts in histology muscle samples of red deer as 'low intensity infection' (<10 sarcocysts per cm^2; [47]), intensity of infection in the heart and diaphragm samples of almost all *Sarcocystis* spp. positive-tested chamois was low. This may be also concluded for the Cantabrian chamois examined by Díez-Baños et al. [25] given that mean sarcocyst counts in 15 small pieces of muscle in chamois and roe deer were 1.9, range 1 to 9 vs. 15.1, range 1 to 155, respectively. In agreement with studies conducted in mouflon and cervids [38,45,47], heart tissue of the Alpine chamois from Germany yielded higher sarcocyst counts than the diaphragm samples.

There are many studies which have found that prevalence and intensity of parasite infections, especially infections with helminths, are higher among male than in female hosts of different taxa of animals; however, male-biased parasitism is not necessarily the general rule as there are several factors, ecological and physiological ones, which may influence the susceptibility and exposure to infection [48]. Regarding sarcocystosis, there are a couple of studies which indicated, at least partly, that male-biased parasitism may play a role for this infection. However, this did not apply to the prevalence of infection but applied only to the intensity of infection measured in terms of sarcocyst counts (but not for all tissues examined in a study) while no difference in the prevalence of infection between male and female animals was found [38,45,46]. This study did not find male-biased sarcocyst counts. In contrast, the diaphragm sarcocyst counts of sexually mature female chamois were significantly higher than the counts in the male animals.

The results of the present study demonstrated that sarcocystosis is highly prevalent in Alpine chamois in Germany but intensity of infections is apparently low. Further studies are desired to

characterize the species of *Sarcocystis* parasitizting chamois using adequate current methodology, in Germany but also in chamois in general, including experimental transmission studies to understand the role of carnivores or other animals in the life cycle of *Sarcocystis* species involving chamois as well as to better understand the circulation of one and the same *Sarcocystis* species in various Caprine hosts (intermediate host-specificity).

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