

THE EUROPEAN BADGER (CARNIVORA : MUSTELIDAE) AS INTERMEDIATE HOST OF FURTHER THREE *SARCOCYSTIS* SPECIES (SPOROZOA)

ODENING K., STOLTE M., WALTER G., BOCKHARDT I.*

Summary :

Three species of sarcocysts are described by light and electron microscopy from the European badger (*Meles meles*) : *Sarcocystis hofmanni* n. sp. (a species otherwise occurring in roe deer, *Capreolus capreolus*), *S. sp.*, cf. *sebeki* (a species usually parasitizing certain murids), and *S. melis* n. sp. (a species presumably specific for the badger).

KEY WORDS : *Meles meles*, *Capreolus capreolus*, *Sarcocystis hofmanni* n. sp., *Sarcocystis sp.*, cf. *sebeki*, *Sarcocystis melis* n. sp., intermediate host specificity.

MOTS CLES : *Meles meles*, *Capreolus capreolus*, *Sarcocystis hofmanni* n. sp., *Sarcocystis sp.*, cf. *sebeki*, *Sarcocystis melis* n. sp., spécificité parasitaire (hôte intermédiaire).

Résumé : LE BLAIREAU EUROPÉEN (CARNIVORA : MUSTELIDAE), HÔTE INTERMÉDIAIRE DE TROIS ESPÈCES SUPPLÉMENTAIRES DE *SARCOCYSTIS* (SPOROZOA).

Trois espèces de sarcocystes sont décrites au microscope optique et électronique chez le blaireau européen (*Meles meles*) : *Sarcocystis hofmanni* n. sp. (espèce par ailleurs rencontrée chez le chevreuil, *Capreolus capreolus*), *S. sp.*, cf. *sebeki* (espèce parasitant habituellement certains muridés), et *S. melis* n. sp. (espèce probablement spécifique du blaireau).

INTRODUCTION

Among others the following questions are connected with findings of sarcocysts in carnivores : Are there some less known predator-prey relations, in which carnivores act as totally normal intermediate hosts of *Sarcocystis*? Or can carnivores be hosts also of sarcocysts, which otherwise ("usually") are found in herbivores, thus breaking the intermediate host specificity which as a rule is considered as being narrow. Quite recently the first record of sarcocysts from the European badger has been published (Odening *et al.*, 1994), which very likely refers to a species usually parasitizing roe deer. Subsequent to this unusual finding three further *Sarcocystis* species have been found in badgers (for survey see Table I), which are described and discussed in the following. Again one of these species is very similar to (and considered identical with) a species of roe deer, another is similar to a species from small mammals, whereas the third shows peculiar features not yet described.

MATERIALS AND METHODS

From September 1992 to September 1993 nine European badgers (*Meles meles*) were investigated, all of them victims of traffic accidents

in Land Brandenburg and Berlin. Seven of them showed slight infection with *Sarcocystis* in the musculature (Table I). Samples of muscle tissue from tongue, diaphragm, oesophagus, heart and skeletal musculature (thigh, loin, thorax, ribs) were investigated, usually in the fresh state. The sarcocysts found individually in the musculature were extracted from the muscular fibres under a dissecting microscope for fresh-state examination and/or processed for histological and transmission electron microscopical (TEM) investigations. For histological investigation 3-5 μ m paraffin sections were made and stained with H/E. A 4 % solution of formaldehyde was used for fixation. Using the semithin sections or fresh preparations, the size of the bradyzoites (cystozoites) was determined. The length of the bradyzoites was taken by measuring the more or less bent median line from pole to pole. The width was measured at the widest diameter.

For TEM investigation the sarcocysts were fixed according to Pospischil and v. Bomhard. After repeated washing with 0.1 mol phosphate buffer, they were post-fixed or pre-contracted in 2% osmium tetroxide solution, dehydrated in ethanol and embedded in Epon 812. After polymerisation for 3 days semithin and ultrathin sections were made. The semithin sections were stained after Richardson. The TEM investigations were carried out using an EM 902 A (Zeiss).

* Institute for Zoo Biology and Wildlife Research, PF 1103, D-10252 Berlin, Germany.

BADGER N°	SARCOCYSTIS SPECIES
1	<i>S. sp.</i> Odening <i>et al.</i> , 1994, cf. <i>gracilis</i> Rätz, 1909 <i>sensu</i> Erber <i>et al.</i> (1978)
2	<i>S. hofmanni</i> n. sp. ; <i>S. sp.</i> , cf. <i>sebeki</i> (Tadros and Laarman, 1976)
3	<i>S. sp.</i> , cf. <i>sebeki</i> (Tadros and Laarman, 1976)
4	<i>S. melis</i> n. sp.
5	negative
6	<i>S. sp.</i> , cf. <i>sebeki</i> (Tadros and Laarman, 1976)
7	negative
8	negative
9	<i>S. sp.</i> , cf. <i>sebeki</i> (Tadros and Laarman, 1976)

Table I. – Survey of single and mixed infection of European badgers from Land Brandenburg and Berlin between September 1992 and September 1993.

DESCRIPTION AND DISCUSSION OF THE SPECIES

SARCOCYSTIS HOFMANNI N. SP. (Figs. 1-3)

Synonyms

Sarcocystis sp. "type Rh 1" Bergmann and Kinder, 1976.
Sarcocystis sp. "thick-walled cyst" Schramlová and Blažek, 1978.
Sarcocystis sp. Erber *et al.*, 1978.
Sarcocystis sp. "type 1, 2, 3" Entzeroth, 1982.
Sarcocystis gracilis in Entzeroth (1985) and Dubey *et al.* (1989), *nec* Rätz, 1909.

Derivatio nominis

The name of the new species is a dedication to Professor Dr. Reinhold R. Hofmann, to whom wildlife biology is greatly indebted for his research work, namely in roe deer and European badger.

Place

Near Fürstenberg, about 70 km NNW of Berlin.

Host

N° 2 : Female ; 11.8 kg in weight ; dead on September 7, 1992 ; deep-frozen ; post-mortem examination on February 19, 1993

Localisation

Heart muscle

Description

The sarcocysts were 0.8-1.2 mm long in fresh state and 78 µm wide in formalin-fixed histological sections. The bradyzoites were elongated with semicircular poles, in part curved ; in semithin section they were 8.3-9.9µm

long ($\bar{x} = 9.3 \mu\text{m}$, $s = 0.61$) and 2.3-3.6 µm wide ($\bar{x} = 2.3 \mu\text{m}$, $s = 0.30$) ; in the transmission electron micrographs their size was 9.4-11.9 µm x 2.3-3.6 µm.

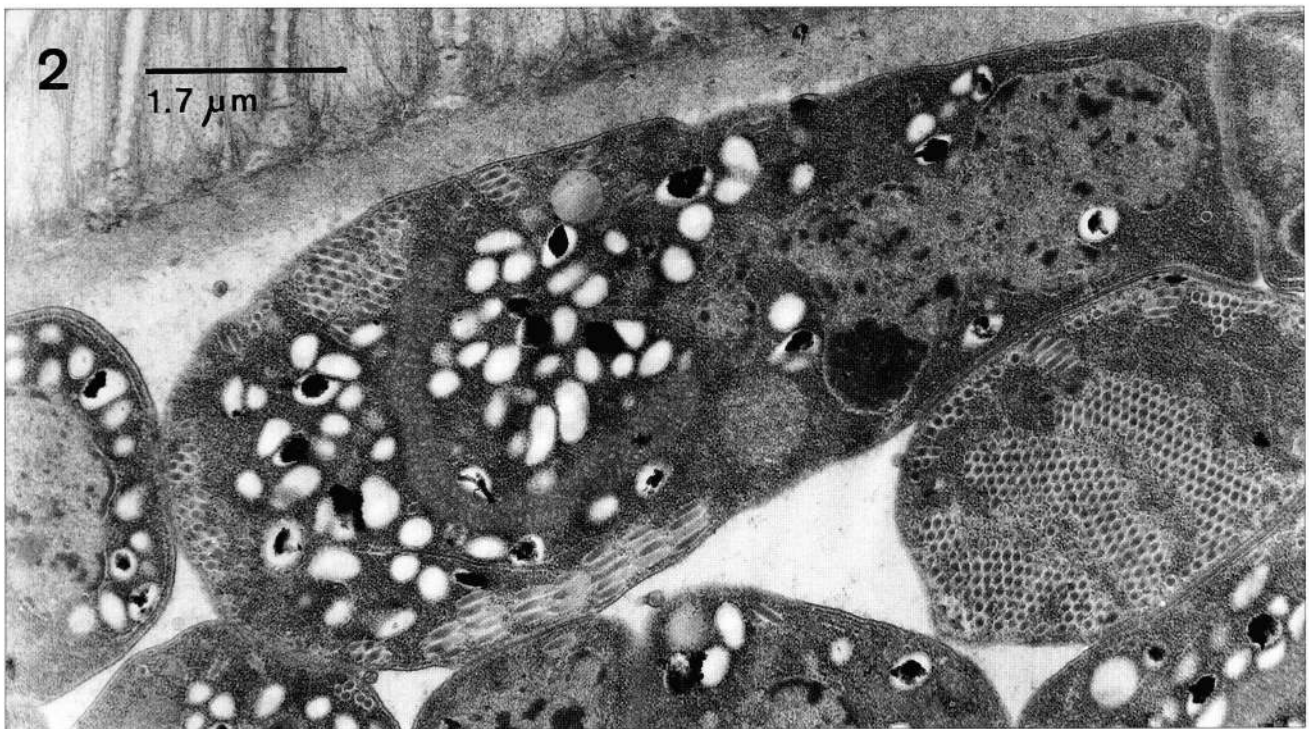
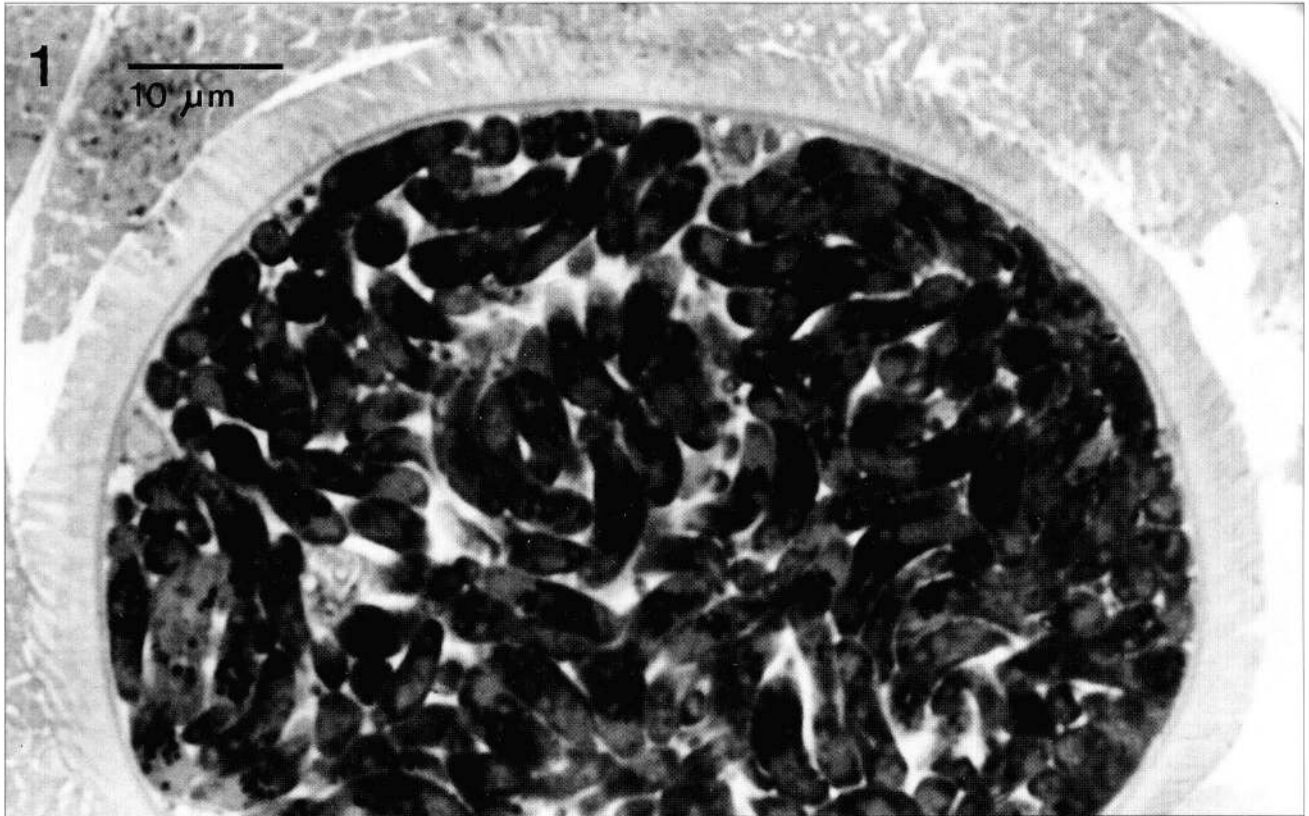
TEM results : Cyst wall (without protrusions) 0.7-0.9 µm wide. The surface of the cyst wall showed palisade-like arranged finger-shaped protrusions. Their diameter was 1.1-1.4 µm at the base, their length 6.3-6.6 µm. The distance between the protrusions was 0.2-0.3 µm at their base. The core of the protrusions consisted of a granular substance, streaked with numerous filaments. The small elevations and fossule-like invaginations with underlying osmiophilic layer covered the space between the finger-shaped protrusions. They were distinctly visible in the lower (proximal) third of the protrusions in our preparations. The protrusions broadly arose from the cyst wall surface. They were rhombic to triangular in cross-section.

Holotype

The specimen depicted in Fig. 2. Collection of Protozoa, Institute for Zoo Biology and Wildlife Research, Alfred-Kowalke-Str. 17, D-10315 Berlin, Germany, No. B 2/1993 (part of a sarcocyst embedded in Epon)

Discussion

We regard the European badger as a devious intermediate host of a species in this case normally using roe deer as intermediate host. We name this species here as a new one (*S. hofmanni* n. sp.), since it has no valid name either as a parasite of roe deer. Rätz (1909) described a *Sarcocystis* species from roe deer as *S. gracilis*. This name is connected with a complicated history. Babudieri (1932), in a review paper on *Sarcocystis*, erroneously ascribed red deer (*Cervus elaphus*) as host to this species. As an outcome of this mistake Machul'skij (1947) and Levchenko (1963) described new *Sarcocystis* species from roe deer, *Capreolus capreolus*, in Central Siberia (*S. sibirica*)



Figs. 1, et 2. – *Sarcocystis bofmanni* n. sp. from *Meles meles*.

1. Semithin section (cross-section) of a mature sarcocyst with cystozoites, showing the palisade-like cyst wall. Stain after Richardson
2. TE micrograph of cystozoites.

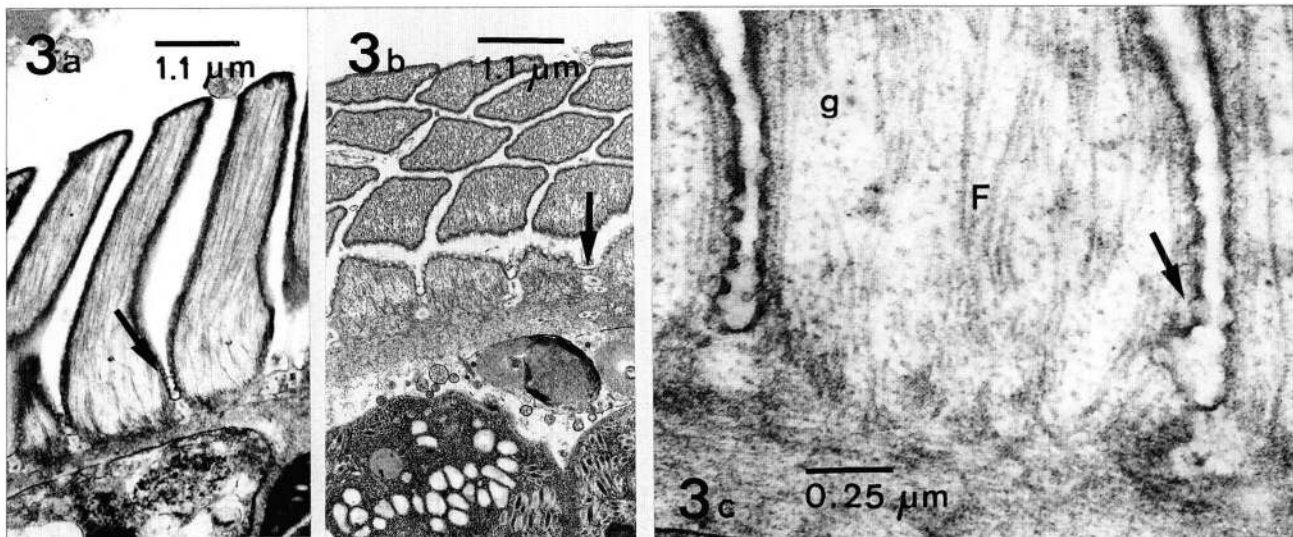


Fig. 3. – *Sarcocystis bofmanni* n. sp. from *Meles meles*.

3. TE micrographs of the finger-shaped protrusions with small elevations on the surface (arrows). The protrusions appear in the cross-section as rhombs (b). Core of the protrusions with many fine granula (g) and longitudinally running filaments (F).

and Kirghizia (*S. capreoli*), because they believed there would not have been described any species of *Sarcocystis* from *Capreolus* yet. The incorrect ascription of the host for *S. gracilis* was repeated also by Kalyakin and Zasukhin (1975) and Levine and Tadros (1980) in important review papers on *Sarcocystis* and corrected only by Levine (1986, 1988) (as a mistake of the summarizing literature). In the original literature this mistake was not made (with the exception of Blažek *et al.*, 1978). There, however, another faulty development took place instead. Erber *et al.* (1978) stated 3 species of *Sarcocystis* in roe deer in Europe, already distinguishable by light microscopy (meanwhile 4 species are known to occur in roe deer). They established one of these species, completely justified, as *S. gracilis* [according to article 17 (2) of the International Code for Zoological Nomenclature, ICZN], named another one as new (*S. capreolicanis*) and designated the third in "open nomenclature" as *S. sp.*, because they could not ascertain the definitive host. After that Entzeroth (1985) designated this third species (*S. sp.*) as *S. gracilis*, without any reference to the decision by Erber *et al.* (1978). Unfortunately, Dubey *et al.* (1989) followed this proceeding. The establishment of *S. gracilis* by Erber *et al.* (1978) was definite (in Rátz, 1909 obviously the figure and the Hungarian text, i. e. the full text, refer to different species, at which the decision by Erber *et al.*, 1978 relates to the figure). Moreover, it was completed by the reference to the ultrastructure of the sarcocyst wall in the presentation by Bergmann and Kinder (1976), which is diagnostically important in the distinction of species (it refers to type 10 of the cyst wall ultrastructure according to the classification by Dubey *et al.*, 1989). A renewed subsequent ascription of establishments once made is not admissible [provided these establishments were made in the sense of article 17 (2) of ICZN].

Erber *et al.* (1978) did not name *Sarcocystis* sp. or "type 3" from roe deer which is morphologically not discernable from *S. bofmanni* n. sp., because they believed that establishing of the definitive host would be necessary for the description of a new *Sarcocystis* species (which is, however, not true).

SARCOCYSTIS SP., CF. *S. SEBEKI* (TADROS AND LAARMAN, 1976) (Figs. 4-5)

Places

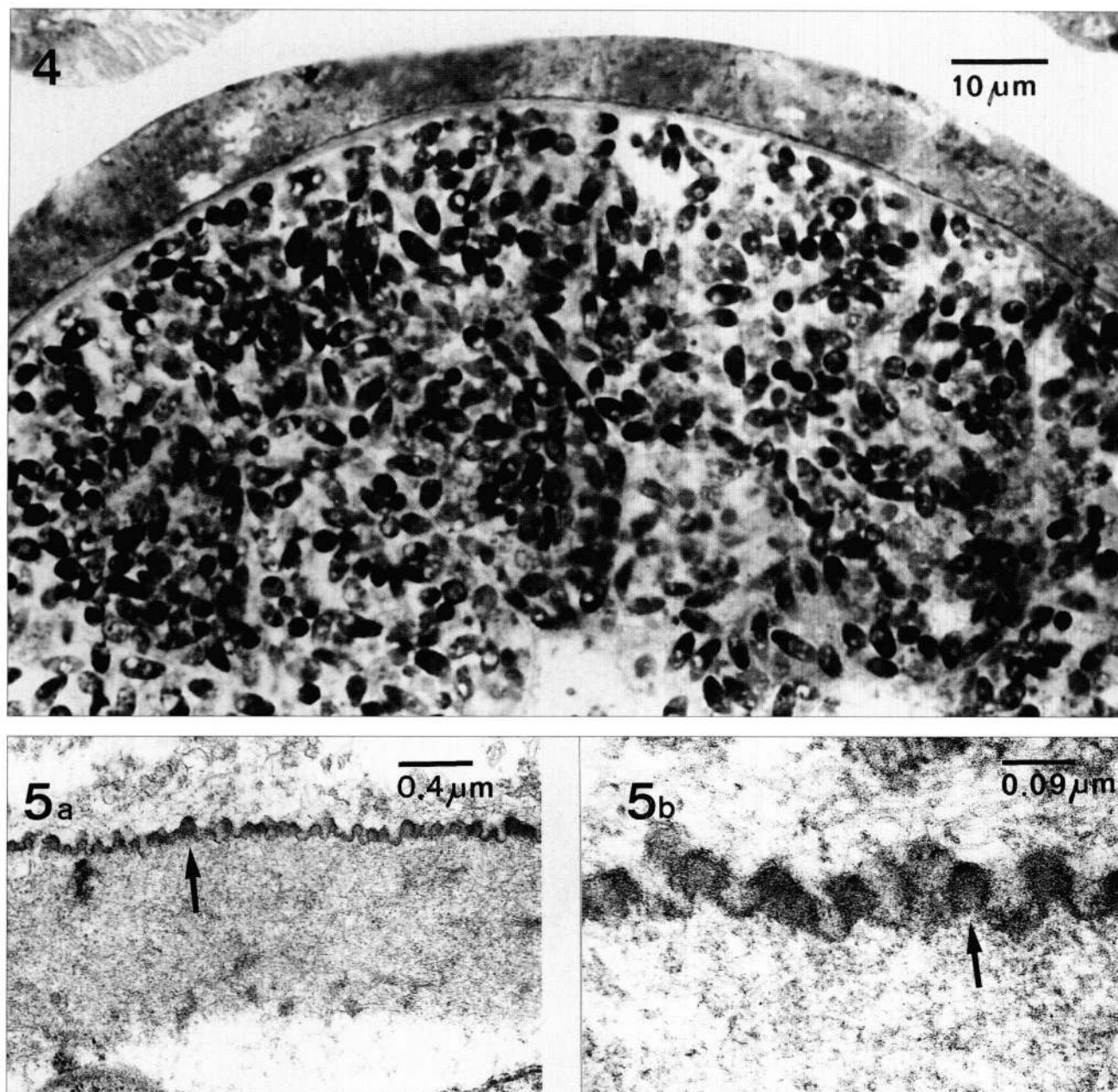
Rhinow, about 75 km WNW of Berlin (badger n° 3)
Near Fürstenberg, about 70 km NNW of Berlin (badger n° 2)
Moncaprice, about 45 km NNW of Berlin (badger n° 6)
Milmersdorf, about 70 km NNO of Berlin (badger n° 9)

Hosts

N° 3 : Male ; 11.5 kg in weight ; dead on June 5, 1992 ; deep-frozen ; post-mortem examination on February 19, 1993
N° 2 : Female, 11.8 kg in weight ; dead on September 7, 1992 ; deep-frozen, post-mortem examination on February 19, 1993
N° 6 : Female, 11 kg in weight, dead on June 6, 1993 ; deep-frozen ; post-mortem examination on July 28/29, 1993
N° 9 : Female, 17.8 kg in weight ; dead on August 31, 1993 ; deep-frozen ; post-mortem examination on September 24, 1993

Localisation

Thigh, loin, thorax, tongue



Figs. 4, 5. – *Sarcocystis* sp., cf. *sebeki* from *Meles meles*.

4. Semithin section (cross-section) of a mature sarcocyst with cystozoites, showing a "thin" cyst wall and empty compartments in the centre. Stain after Richardson. 5. TE micrographs of the cyst wall with the small elevations on the surface (arrows). Substance in the core of the small elevations more compact than the ground substance of the cyst wall.

Description

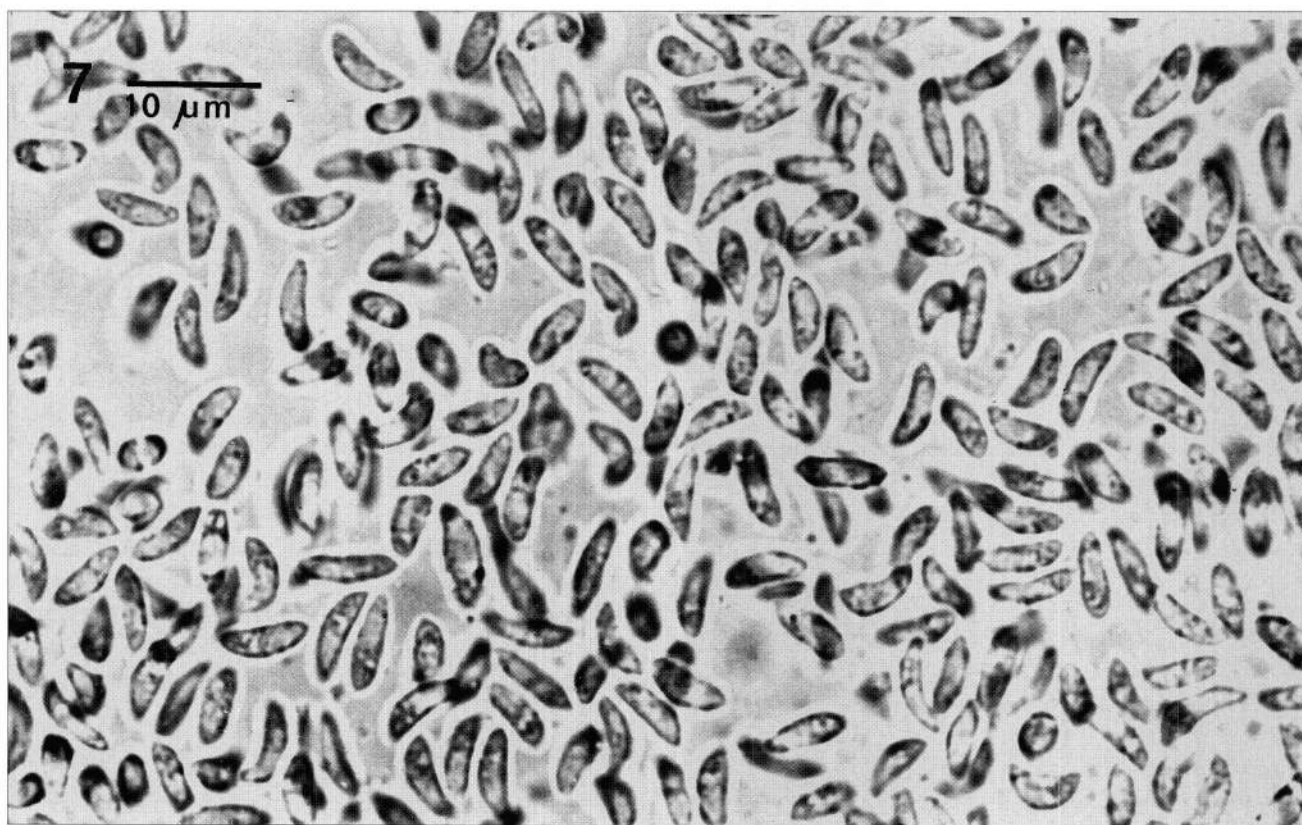
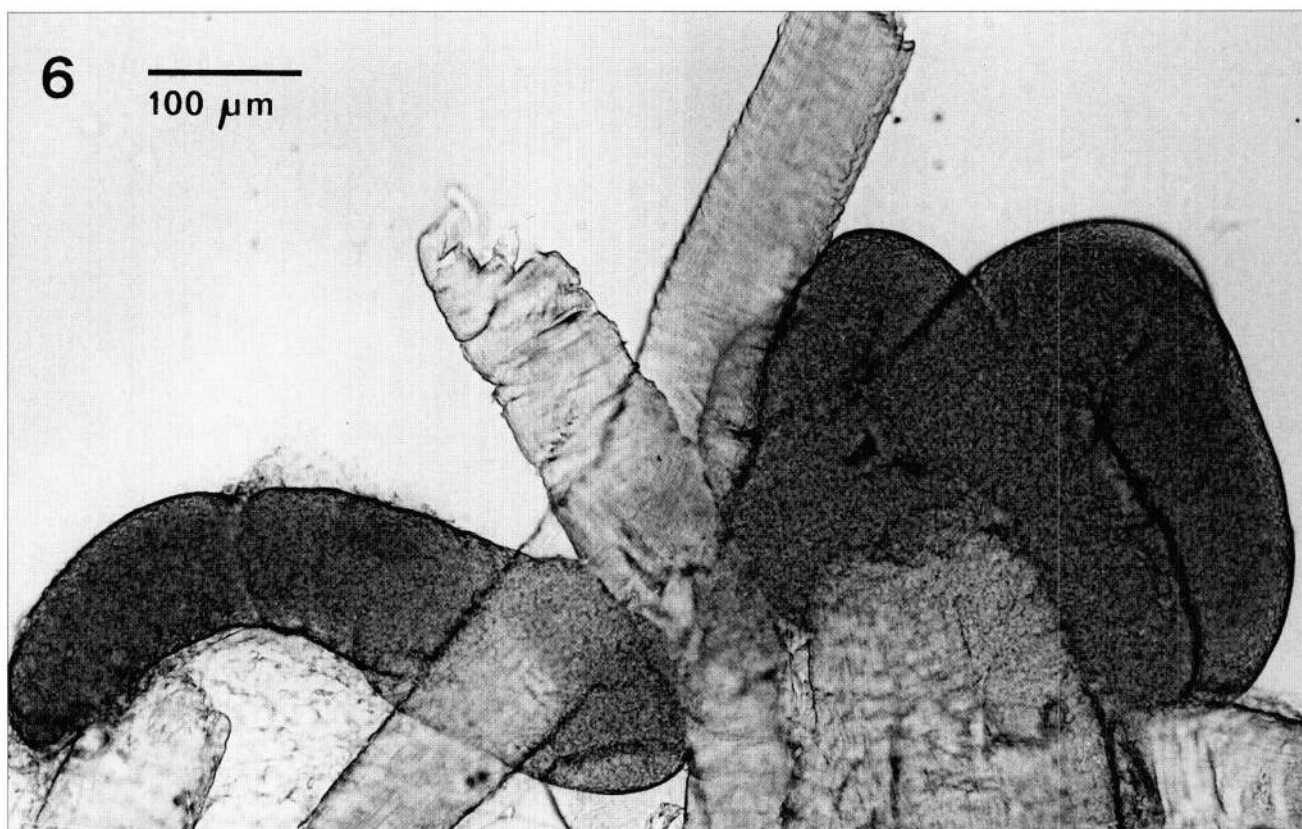
Sarcocysts 6.5-9.0 mm long and 172-200 μm wide in fresh state. Bradyzoites squatly fusiform, in fresh state 6.1-7.2 μm long ($\bar{x} = 6.9$ μm, $s = 0.26$) and 1.6-2.2 μm wide ($\bar{x} = 1.9$ μm, $s = 0.24$); in semithin section 5.7-6.3 μm long ($\bar{x} = 5.9$ μm, $s = 0.29$) and 1.7-2.2 μm wide ($\bar{x} = 2.0$ μm, $s = 0.16$).

TEM results: The cyst wall was 0.9-1.4 μm wide. There were no protrusions. The small elevations of the primary cyst wall were 0.08-0.09 μm long, the fossule-like invaginations in between had a maximum diameter of

0.05 μm. Their distance from each other was about 0.05 μm. The small elevations and invaginations were underlaid with an osmiophilic layer. The ground substance (granular layer) of the cyst wall was fine-grained and condensed within the small elevations.

Discussion

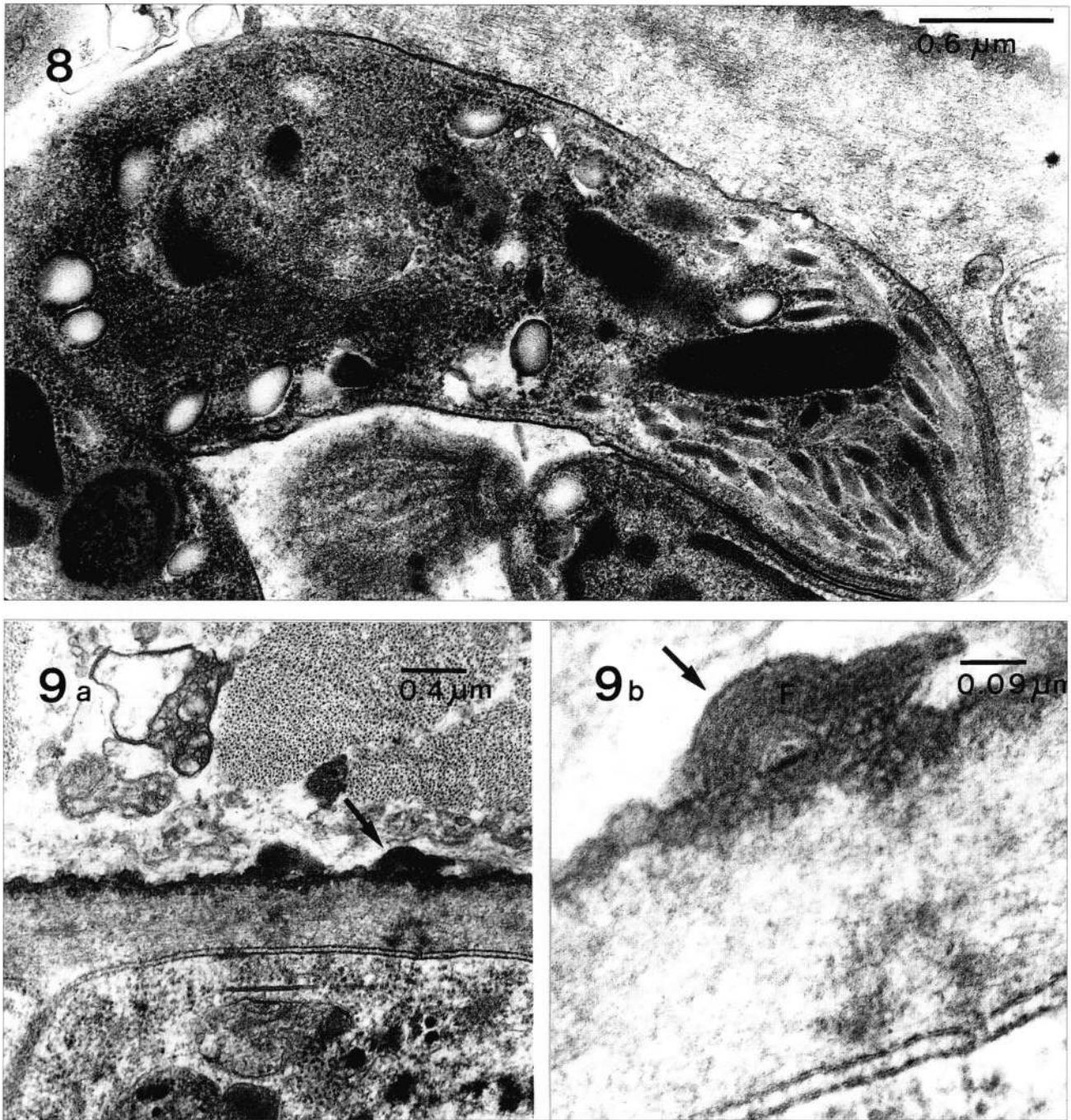
This species shows type 1 of the cyst wall ultrastructure (according to the classification by Dubey *et al.*, 1989). We assign it near *S. sebeki* (cf. Tadros and Laarman 1976, 1978, 1979, 1980, 1982), because it is



Figs. 6, 7. – *Sarcocystis melis* n. sp. from *Meles meles*.

6. Two intertwined sarcocysts in the fresh state, for the most part extracted from the muscular fibres

7. Released cystozoites in the fresh state.



FIGS. 8, 9. – *Sarcocystis melis* n. sp. from *Meles meles*.

8. TE micrograph of a bradyzoite.

9. TE micrographs of the cyst wall with the filiform protrusions (arrows), which are streaked with parallel running fibrillary elements (F).

morphologically very similar to this species and because it was already earlier supposed that *S. sebeki* could infect *Mustela nivalis* as well, a mustelid, beside its “normal” intermediate hosts (*Apodemus sylvaticus* and *Mus musculus*; the definitive host is *Strix aluco*) (Tadros and Laarman, 1979). Tadros and Laarman (1979) found sarcocysts in the musculature of a European weasel, described them by light microscopy and fed them to a tawny owl, in which a weak infection was obtained.

SARCOCYSTIS MELIS N. SP. (Figs. 6-9)

Derivatio nominis

Genitive singular of *meles* (Latin = badger)

Place

Berlin (Grunewald)

Host

N° 4 : Female ; 8 kg in weight ; dead on March 3, 1993 ; post-mortem examination on March 22, 1993.

Localisation

Loin

Description

Sarcocysts 2-5 mm long in fresh state and 51-71 μm wide in histological sections (60 μm in semithin sections). Compartments polyhedral, with maximum 7 sides, 9.3-11.0 μm wide (\bar{x} = 9.6 μm , s = 1.42) and 7.1-13.8 μm deep (\bar{x} = 11.6 μm , s = 2.33). Bradyzoites fusiform, 6.9-8.2 μm long (\bar{x} = 7.4 μm , s = 0.54) and 0.8-1.7 μm wide (\bar{x} = 1.4 μm , s = 0.28) in semithin sections.

TEM results: Cyst wall 0.4-0.6 μm wide. Small "elevations" and fossule-like invaginations (diameter about 0.05 μm) of the primary cyst wall poorly pronounced. The primary cyst wall consisted proximally (internally) of an osmiophilic layer. Peculiarly formed filiform protrusions arose from the surface in irregular, mostly greater distances. They lay close to the primary cyst wall or overlapped it. One of them, well visible, was 0.5 μm long and at its base 0.07 μm wide. The filiform protrusions were streaked with several parallel, longitudinally running fibrillar elements, which partially proceeded into the ground substance of the cyst wall.

Holotype

The specimen lying behind Fig. 8. Collection of Protozoa, Institute for Zoo Biology and Wildlife Research, Alfred-Kowalke-Str. 17, D-10315 Berlin, Germany, No. B 3/1993 (part of a sarcocyst embedded in Epon).

Discussion

S. melis n. sp. has an ultrastructure of the cyst wall hitherto not described (cf. Dubey *et al.*, 1989). Therefore, it could refer to a species specific to the European badger.

It could be that the characteristic filiform protrusions are longer than stated in our description, if our figure refers to first cuts of folded protrusions, or if longer hair-like formations should be broken off. Both would not call into question the peculiarity of the structures described.

REFERENCES

- Babudieri B. : I sarcosporidi e le sarcosporidiosi. (Studio monografico). *Arch. Protistenkd.*, 1932, 76, 421-580.
- Bergmann V., Kinder E. : Elektronenmikroskopische Untersuchungen zur Wandstruktur von Sarkozysten in der Skelettmuskulatur von Wildschwein und Reh. *Mon.b. Vet.med.*, 1976, 31, 785-788.
- Blažek K., Schramlová J., Ippen R.: Dog as definitive host of Sarcosporidia infecting roe deer. *Fol. Parasitol. (Praha)*, 1978, 25, 95-96.
- Dubey J. P., Speer C. A., Fayer R. : Sarcocystosis of animals and man. *CRC Press, Inc.*, Boca Raton, Florida, 1989, 215 p.
- Entzeroth R. : A comparative light and electron microscope study of the cysts of *Sarcocystis* species of roe deer (*Capreolus capreolus*). *Z. Parasitenkd.*, 1982, 66, 281-292.
- Entzeroth R. : Light-, scanning-, and transmission electron microscope study of the cyst wall of *Sarcocystis gracilis* Rätz, 1909 (Sporozoa, Coccidia) from the roe deer (*Capreolus capreolus* L.). *Arch. Protistenkd.*, 1985, 129, 183-186.
- Erber M., Boch J., Barth D. : Drei Sarkosporidienarten des Rehwildes. *Berl. Münch. Tierärztl. Wochenschr.*, 1978, 91, 482-486.
- Kalyakin V. N., Zasukhin D. N. : Distribution of *Sarcocystis* (Protozoa: Sporozoa) in vertebrates. *Fol. Parasitol. (Praha)*, 1975, 22, 289-307.
- Levchenko N. G. : A case of sarcosporidiosis in roe deer *Capreolus capreolus* [in Russian]. *Trudy Instituta Zoologii Akad. Nauk Kazakh. SSR (Alma-Ata)*, 1963, 19, 244-245.
- Levine N. D. : The taxonomy of *Sarcocystis* (Protozoa, Apicomplexa) species. *J. Parasitol.*, 1986, 72, 372-382.
- Levine N. D.: The protozoan phylum Apicomplexa. Vol. II. *CRC Press, Inc.*, Boca Raton, Florida, 1988, 154 p.
- Levine N. D., Tadros W. : Named species and hosts of *Sarcocystis* (Protozoa : Apicomplexa: Sarcocystidae). *Syst. Parasitol.*, 1980, 2, 41-59.
- Machul'skij S. N.: Sarcosporidiosis of wildlife in Buryat-Mongolia [in Russian]. *Trudy Buryat-Mongol. Zoovet. Inst.*, 1947, 3, 87-92.
- Odening K., Stolte M., Walter G., Bockhardt I., Jakob. W.: Sarcocysts (*Sarcocystis* sp.: Sporozoa) in the European badger *Meles meles*. *Parasitology*, 1994, 108, in press.
- Rätz I. : Die Sarcosporidien und ihre in Ungarn vorkommenden Arten [in Hungarian]. *Állattani Közlemények*, 1909, 8, 1-37, Tab. I-III ; 91-95 [German review].
- Schramlová J., Blažek K.: Ultrastruktur der Cystenwand der Sarkosporidien des Rehes (*Capreolus capreolus* L.). *Z. Parasitenkd.*, 1978, 55, 43-48.
- Tadros W., Laarman J. J. : *Sarcocystis* and related coccidian parasites: a brief general review, together with a discussion on some biological aspects of their life cycles and a new proposal for their classification. *Acta Leidensia*, 1976, 44, 1-107.
- Tadros W., Laarman J. J.: A comparative study of the light and electron microscopic structure of the walls of the muscle cysts of several species of sarcocystid eimeriid Coccidia. *Proc. K. Nederl. Akad. Wet., Ser. C*, 1978, 81, 469-491.
- Tadros W., Laarman J. J.: Muscular sarcosporidiosis in the common European weasel, *Mustela nivalis*. *Z. Parasitenkd.*, 1979, 58, 195-200.
- Tadros W., Laarman J. J. : Further investigations on the life history and transmission of *Sarcocystis sebeki* of *Apodemus sylvaticus*. *Trop. Geograph. Med.*, 1980, 32, 362.
- Tadros W., Laarman J. J.: Current concepts on the biology, evolution and taxonomy of tissue cyst-forming eimeriid Coccidia. *Adv. Parasitol.*, 1982, 20, 293-468.

Accepted le 2 décembre 1993