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Vipera berus and *V. ammodytes* (Serpentes: Viperidae) represent new hosts for *Caryospora simplex* (Apicomplexa: Eimeriidae) in Europe

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Abstract. During a survey of the coccidian parasites of reptiles, caryosporan oocysts were found in the faeces of wild and captive European viperid snakes *Vipera berus* (L.) and *V. ammodytes* (L.). Thirty two of 37 examined *V. berus* (86%) and 9 of 17 examined *V. ammodytes* (53%) specimens were found to be passing caryosporan oocysts. Morphological characters of all caryosporan isolates were identical and fitted well with the description of *Caryospora simplex* Léger, 1904. Experimental inoculation of severe combined immunodeficient (SCID) mice with seven isolates of *C. simplex* from *V. berus* or *V. ammodytes* confirmed the heteroxenous life cycle pattern, for the first time for isolates of evidently European origin. Caryosporan developmental stages were observed in the connective tissues of the nose, cheeks, ear and scrotum in all inoculated SCID mice. *V. berus* and *V. ammodytes* represent new hosts for *C. simplex*. The present paper represents the first widely based report on coccidian parasites of the genus *Caryospora* Léger in European viperids. Our findings indicate a wide distribution of *C. simplex* throughout the range of distribution of snakes of the genus *Vipera*.

Coccidian species of the genus *Caryospora* Léger (Apicomplexa: Eimeriidae) are usually found in reptiles and birds and are characterized by monosporocystic, octozoic oocysts. Some caryosporan parasites have facultatively heteroxenous life cycle (Cawthorn and Stockdale 1982, Wacha and Christiansen 1982, Upton et al. 1984a).

Caryospora simplex Léger, 1904, first reported as *Karyospora simplex* (by printing error), was described from the intestine of naturally infected *Vipera aspis* in France (Léger 1904). Developmental stages of *C. simplex* were first described by Léger (1911) in the intestinal epithelium of naturally infected *V. aspis*. Galli-Valerio (1929) reported this parasites also from *V. aspis* from France. Lavier (1939) reviewed the endogenous development in *V. aspis* and attempted unsuccessfully to transmit the parasite to three young *V. aspis* by oral inoculation of oocysts.

This coccidian species was later redescribed by Upton et al. (1983) from captive Ottoman viper *Vipera xanthina xanthina* and was also found in captive Russell's viper *Daboia* (= *Vipera*) *russelli russelli* (Upton et al. 1986). Upton and Barnard (1986) successfully transmitted *C. simplex* from *V. xanthina* to Palestine viper *V. palestinae*. Slightly different morphologic type was found in wild Kaznakov's viper *Vipera kaznakovi* from West Caucasus (Matuschka 1986).

Recently, Wilber et al. (1995) detected oocysts and developmental stages of *C. simplex* in captive *V. kaznakovi* in USA. Authors of papers, published since 1939, worked with isolates of non-European origin. Surprisingly, this unusual coccidia was not described to date from any other European viperid except *V. aspis* from France.

The main aim of our study was to find coccidia of the genus *Caryospora* in two European viperids – *Vipera berus* (L.) and *V. ammodytes* (L.) and to confirm or exclude their conspecifity with *C. simplex* from previous studies. In several experimental trials was, for the first time, tested the ability of European isolates of *C. simplex* to cause dermal caryosporosis in mammalian hosts.

MATERIALS AND METHODS

The following viperid snakes were examined coprologically for the presence of coccidian parasites:

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a) **Captured snakes**. Fifteen specimens of *Vipera berus berus* (Linnaeus, 1758) were collected in a period 1994–1996 from 4 localities in the Czech Republic. Individual localities are listed in Table 1. *Vipera ammodytes montandoni* Boulenger, 1904: two specimens were collected in SE Bulgaria in 1995.

All collected snakes were placed individually in small plastic cages and transported into the laboratory. The snakes were housed individually in 15 l plastic cages at 22–25°C during daytime and 17–20°C during the night. The floor of the cages was covered with paper towels and locally heated by heating pad to 27°C during the day. The snakes were fed laboratory raised common voles (*Microtus arvalis*) and laboratory mice. The faecal samples were collected from the ground of cages. Individual faecal samples were collected repeatedly 2–5 times from each snake and then the snakes were released in the original localities.

b) **Faecal samples**. The faecal samples of 20 specimens of *Vipera berus berus*, submitted by C. Wild and C. Entwistle, were collected during the ecological field study in Beetley Common, Dereham – Norfolk, England. Fresh faecal samples were fixed with 70% ethanol and shipped to the Czech Republic.

c) **Captive snakes**. The faecal samples from two specimens of *Vipera b. berus* and 15 specimens of *V. ammodytes* ssp. (subspecific status not determined) were obtained from different herpetoculturists in the Czech Republic. The origin of these snake (if known) is listed in Table 1.

Faecal samples were screened routinely for parasites using flotation in Sheather's sugar solution (specific gravity 1.30). Faecal samples containing unsporulated oocysts were placed in Petri dishes with a thin layer of 2.5% potassium dichromate solution and were incubated at 25–27°C for two weeks. Fifty sporulated oocysts were measured using bright-field microscopy (100 × objective) with a calibrated ocular micrometer to obtain morphologic data. All measurements are given in micrometers (μm), given as the mean followed by range in parentheses. Isolated oocysts were also examined and photographed using Nomarski interference contrast (NIC) microscopy. Faecal samples containing coccidian oocysts were resuspended with fresh potassium dichromate solution, filtered and stored at 4°C before further use.

In order to confirm heteroxenous development, severe combined immunodeficient (SCID) mice were used for experimental inoculations. SCID mice were housed in flexible film isolators (BEM, Znojmo; Czech Republic) with high-efficiency particulate air (HEPA) filters. All cages, food, water, and bedding were sterilized before use. A total of 20 eight to eleven-week-old SCID mice of both sexes were used in experimental trials with four caryosporan isolates from *V. berus* and three caryosporan isolates from *V. ammodytes* (see Table 1). Prior to use, sporulated oocysts were disinfected in 50% commercial bleach (SAVO, Bochemie, Czech Republic) for 5 min and washed three times by centrifugation in a sterile phosphate-buffered solution (PBS). SCID mice were perorally (p.o.) inoculated with 10^2–10^3 sporulated oocysts in a single 1 ml volume. All mice were monitored daily for clinical signs of disease or death due to caryosporosis. On different days post inoculation (DPI) moribund mice were euthanized with ether and necropsied. At necropsy, tissue samples of the stomach, duodenum, middle jejunum, ileum, caecum, colon, rectum, heart, lung, liver, kidney, urinary bladder, dermal connective tissues from the cheeks, tongue, nose, ears, scrotum from males and vagina from females, and left *biceps femoris* muscle were fixed in 10% buffered formalin. Fixed tissues were processed for light microscopy using standard methods. Paraffin sections were stained with hematoxylin and eosin (HE) and Giemsa.

Table 1. Results of coprological examinations of individual *Vipera* specimens and tests of heteroxenity of *Caryospora simplex* isolates from individual localities.

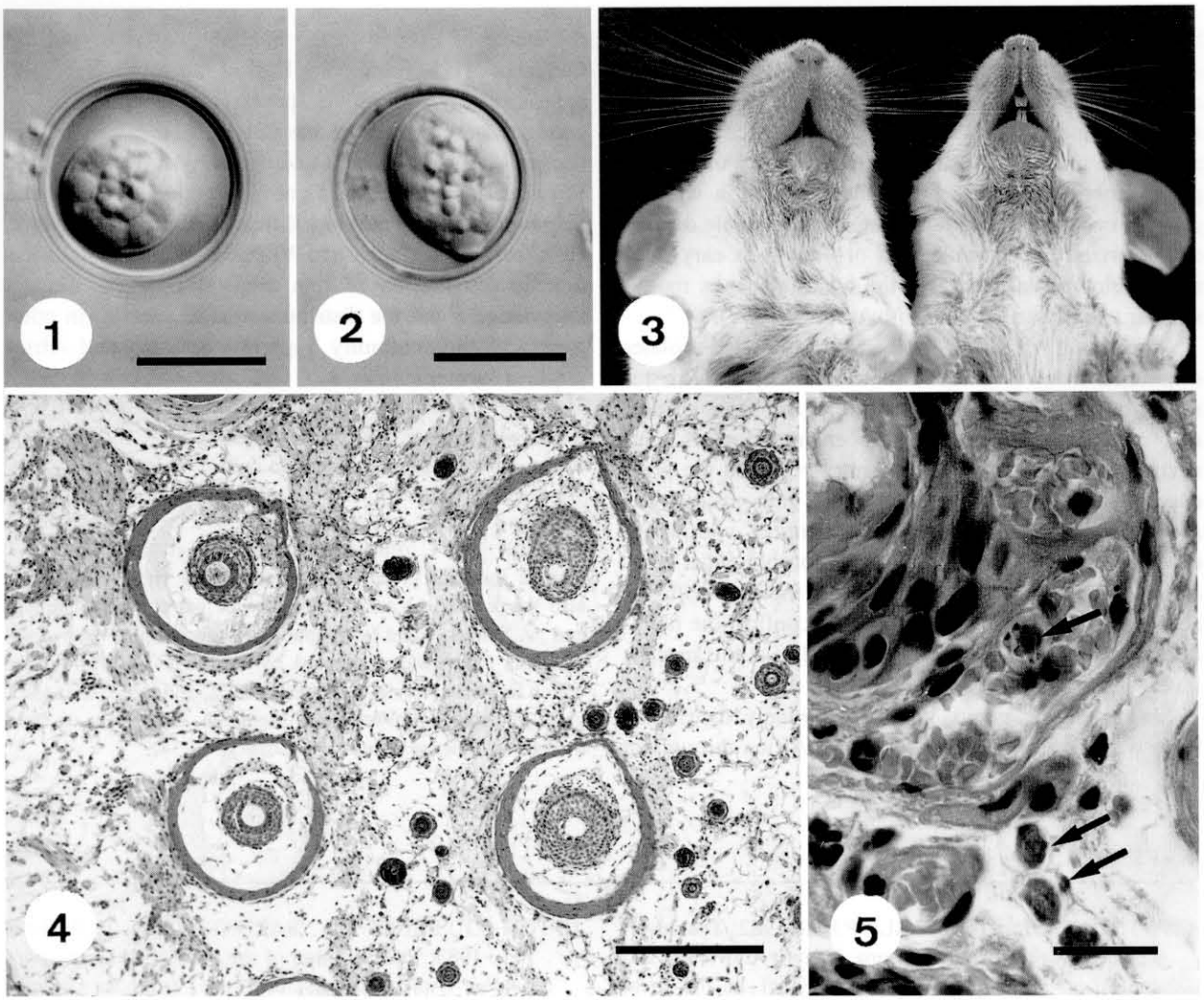
Host species	Origin	No. examined	Prevalence (%)
Vipera ammodytes	Albania (captive)	1	0
	Croatia (captive)	1	0
	Tsarevo, Bulgaria	2	50*
	captive	10	70*
	captive	1	100*
	captive	2	0
Vipera berus	Hraničná, Czech Republic (CR)	1	100*
	Nové Město na Moravě, (CR)	12	75*
	Desná, Czech Republic	2	100*
	Skalité, Slovak Republic (captive)	2	100*
	Dereham, Norfolk, England	20	90*

* All tested isolates were found to be heteroxenous in experimental trials with SCID mice.

RESULTS

Thirty two of 37 examined *Vipera berus* (86%) and 9 of 17 examined *V. ammodytes* (52%) specimens were found to be passing caryosporan oocysts (see Table 1). The morphological features and measurements of oocysts of all examined isolates were identical. Typical morphological features of sporulated oocysts from *V. berus* and *V. ammodytes* follow.

Oocysts spherical, 13.7 (13.5–15.0) in diameter, with a single-layered oocyst wall, ca. 1.5 thick. Shape index (length/width ratio) of sporulated oocysts 1 : 1. A micropyle and oocyst residuum absent. One globular polar granule, ca. 2.0 in diameter, present. Sporocysts ovoid, 11.0 (10.5–12.0) × 7.8 (7.0–8.5), with smooth, colourless and unilayered sporocyst wall, shape index 1.4 (1.3–1.5). Stieda body present, knob-like, ca. 2.0 high and 1.0 wide, homogenous substieda body present, ca. 2.5 high and 1.0 wide. Sporocyst residuum present as small granules of irregular size scattered among sporozoites. Sporozoites elongate, arranged head to tail within sporocyst. Large anterior and slightly smaller posterior refractile bodies present with nucleus situated between them (Figs. 1,2).



Figs. 1–2. Nomarski interference contrast (NIC) photographs of sporulated oocysts of *Caryospora simplex* from *Vipera berus* from Nové Město na Moravě, Czech Republic. Scale bar = 10 μm. **Fig. 3.** Comparison of SCID mouse experimentally infected with *C. simplex* (20 days post infection (DPI) – left) with the control mice (right), demonstrating swelling of cheeks after ifection. **Fig. 4.** Cheek of SCID mice experimentally infected with *C. simplex* (18 DPI). Inflammatory edema with widely dilated hair follicle. HE. Scale bar = 400 μm. **Fig. 5**. Nose of the SCID mice experimentally inoculated with *C. simplex* (18 DPI). Numerous thin-walled sporulated oocysts in connective tissue (arrows). HE. Scale bar = 20 μm.

All inoculated SCID mice exhibited lethargy associated with rough hair coat and considerable swelling of the facial tissue (Fig. 3), foot pads, scrota or external genitalia of females from 8 days post infection (DPI). In the following days, more pronounced clinical signs with wasting and subsequent mortality were observed. None of inoculated SCID mice survived more than 28 DPI. Histological examination revealed extensive areas of inflammatory edema in skin, subcutis and adjacent muscular tissue mainly in nose and facial tissue, at the base of ears, footpads, in scrotum or external genitalia of females. Inflammatory edema was situated mainly in tactile and other hairs follicles (Fig. 4). Caryosporan developmental stages (Fig. 5) were observed in connective tissue components of the nose, cheeks, ear, scrotum in all SCID mice examined.

DISCUSSION

All examined and measured morphological characters of oocysts were found to be identical with original description of *Caryospora simplex* (Léger 1904) and redescription of this species by Upton et al. (1983). We also did not find morphological differences between individual isolates from *Vipera berus* and *V. ammodytes*. Until now, no other coccidian parasite of the same size range and with exogenous sporulation has been described from viperid snakes. Therefore, coccidian oocysts from faeces preserved with ethanol are also suggested to belong to *C. simplex*, regardless they were unsporulated.

Descriptions of caryosporan coccidia based upon the morphological characters of oocysts has been criticized

by Matuschka (1986). Except for morphological characters of exogenous stages, ability to cause dermal coccidiosis in rodents is suggested to be typical for *C. simplex* (Upton et al. 1984a). The SCID mice represent a valuable model for testing the heteroxenity of caryosporan isolates. In these mice, the inoculation with *C. simplex* oocysts has produced fatal and systemic disease characterized by dissemination of numerous caryosporan developmental stages in the host cutaneous mononuclear phagocyte system (Vítovec et al. 1997).

Inoculation of SCID mice with all tested isolates originated from *V. berus* and *V. ammodytes* have resulted in typical clinical and pathological signs of dermal caryosporosis. Histological examination of connective tissues of all examined mice revealed the presence of endogenous stages – meronts, gamonts, oocysts and caryocysts, morphologically identical with those described by Upton et al. (1984a). Morphological characters of all our caryosporan isolates together with the ability to produce dermal coccidiosis confirm the identity with *C. simplex* from previous studies at the specific level.

Snakes of the genus *Vipera* are widely distributed from western Europe and Northwest Africa throughout the whole of Europe to the Sakhalin eastward and the Caucasus, Turkey and Near East in the south. Recently, approximately 21 *Vipera* species are recognized. Occurrence of *Caryospora simplex* in *V. aspis* in France (Léger 1904, 1911, Galli-Valerio 1929, Lavier 1939), in *V. berus* in England and Central Europe (this study), in *V. ammodytes* in southern Bulgaria (this study) and *V. kaznakovi* from Caucasus (Wilber et al. 1995) indicates a wide distribution of this unusual coccidia. Deeper knowledge about the distribution of *C. simplex* in other genera of the subfamily Viperinae in Asia and Africa requires further studies.

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