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Complete Development of the Porcine Coccidium *Isospora suis* Biester, 1934 in Cell Cultures

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ABSTRACT: Development from inoculated sporozoites to unsporulated oocysts of *Isospora suis* Biester, 1934 is described in a swine testicular (ST) cell line. Sporozoites penetrated ST cells within 1 hr postinoculation (PI). Development was initially by endodyogeny to produce binucleate type I meronts and type I merozoites. Division by endodyogeny continued during the 13-day observation period and type I merozoites were the developmental stages most abundant at observation periods >3 days PI. Mutinucleate type II meronts and type II merozoites were first observed 7 days PI. Gamonts and oocysts were present 12 days PI. Oocysts did not sporulate in vitro. The ultrastructural features of stages were similar to those that occur in the pig host.

Isospora suis Biester, 1934 is the causative agent of neonatal porcine coccidiosis. This disease causes morbidity and mortality in nursing pigs and is a common production problem in the swine industry in the United States and other countries worldwide (see Lindsay et al., 1997).

Fayer et al. (1984) were the first to describe development of *I. suis* in cell cultures. Development was limited to replication by endodyogeny. Lindsay and Blagburn (1987) reported similar findings in primary porcine and bovine kidney cell cultures; however, they did observe a few multinucleate stages that resembled type II meronts (Lindsay et al., 1980) of *I. suis*. The present study reports complete development of *I. suis* from sporozoites to unsporulated oocysts in a swine testicle cell line.

Infection and fixation of cell cultures were conducted at Ambico, Inc., Dallas Center, Iowa. Processing of tissues for transmission electron microscopy (TEM) and TEM observations were done in the Department of Pathobiology, College of Veterinary Medicine, Auburn University, Alabama. Examination of thin sections and electron micrographs were conducted in the Center for Molecular Medicine and Infectious Diseases, Department of Biomedical Sciences and Pathobiology, Virginia–Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, Virginia.

A diploid swine testicle (ST) cell line (Ambico, Inc., Dallas Center, Iowa) was used. The ST cells were grown in minimum essential medium with Earle's salts supplemented with 10% (v/v) bovine calf serum and 100 μg/ml gentamicin. Cells were plated at 1.25×10^6 ST cells per 25-cm^2 cell culture flask. Cultures were incubated at 37 C in an incubator with a 5% CO_2–95% air atmosphere.

Sporozoites of *I. suis* were obtained from sporulated oocysts and separated from oocyst/sporocyst walls as previously described (Lindsay et al., 1983; Lindsay and Current, 1984). Between 1.25 and 5×10^5 sporozoites in 5 ml of inoculation medium were used for infection of ST cell cultures. The inoculation medium consisted of Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% bovine calf serum, 0.1 M HEPES buffer, and 100 μg/ml gentamicin. Sporozoites were allowed 75 min to penetrate ST cells, then any extracellular sporozoites were removed by washing the monolayer 3 times with DMEM. Ten milliliters of the DMEM was then added to the monolayer and they were incubated at 37 C until processed for TEM.

Flasks of infected ST cells were processed for TEM 1 and 22 hr and 3, 4, 5, 7, 10, 12, and 13 days postinoculation (PI). The ST cells were scraped from the plastic growth surface using a cell scraper and pelleted by centrifugation. The cell pellet was fixed in 3% (v/v) glutaraldehyde in Millonig's phosphate buffer (pH 7.4). The pellets were postfixed in 1% (w/v) osmium tetroxide, dehydrated in a series of ethanols, passed through 2 changes of propylene oxide, and embedded in Spurr resin (Polysciences Inc., Warrington, Pennsylvania). Thin sections were stained with uranyl acetate and lead citrate and examined with a Philips 301 transmission electron microscope operating at 60 kV. Additionally, 1-μm-thick sections were placed on glass microscope slides and stained with toluidine blue and examined by light microscopy.

Sporozoites were the only stages observed 1 and 22 hr PI. Division by endodyogeny had occurred by 3 days PI and ST cells with up to 8 type I merozoites were present. Infected ST cells containing type I merozoites were observed at every observation period from days 3 to 13 PI when the last sample was examined and they were always the most abundant stage present. The length of time sporozoites remained could not be determined because sporozoites closely resemble type I merozoites. Type II meronts and type II merozoites were first observed

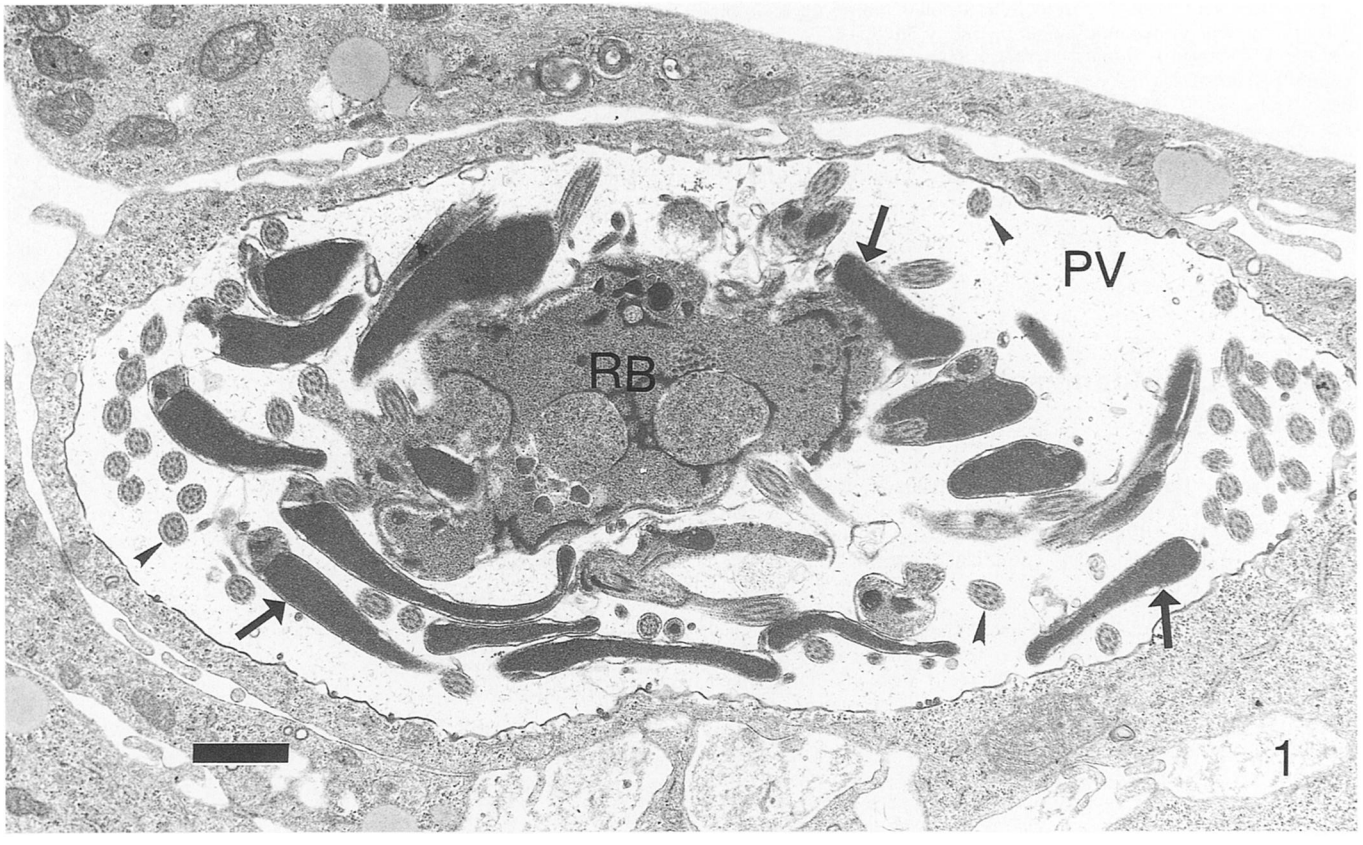

FIGURE 1. Transmission electron micrograph of a microgamont of *Isospora suis* in a swine testicular cell. The microgamont is in a parasitophorous vacuole (PV). Note nuclei (arrows) and flagella (arrowheads) of the microgametes that are arising from a residual body (RB). Bar = 1 μm.

day 7 PI. Sexual stages and oocysts were first seen 12 days PI (Figs. 1, 2). Host cells containing asexual and sexual stages in the same cells were occasionally observed. Oocysts did not sporulate in vitro. No attempts were made to induce sporulation of in vitro-produced oocysts. Continuous culture of *I. suis* was not achieved.

The ultrastructural features of the endogenous stages of *I. suis* in ST cells are similar to those described in experimentally infected pigs (Matuschka and Heydorn, 1980; Matuschka, 1982), for sporozoites and type I meronts and merozoites of *I. suis* in cell cultures (Lindsay et al., 1991), and in vitro-excysted *I. suis* sporozoites (Pinckney et al., 1993). The timing of appearance of type II meronts and merozoites and sexual stages was delayed in vitro over that observed in pigs (Lindsay et al., 1980; Matuschka and Heydorn, 1980).

Lindsay and Current (1984) obtained complete development of *I. suis* in the chorioalantoic membrane of chicken embryos. However, previous attempts to obtain complete development of mammalian *Isospora* species in cell cultures have been unsuccessful (Fayer, 1972; Fayer and Mahrt, 1972; Fayer and Thompson, 1974; Fayer et al., 1984; Lindsay and Blagburn, 1987). The vast majority of *Eimeria* species examined in vitro complete only 1 or 2 asexual generations.

Many factors influence development of coccidian parasites in vitro (see Strout and Schmatz, 1990; Upton, 1997), and it is often difficult or seemingly impossible to find the precise conditions needed for a particular species. *Eimeria tenella, Cryptosporidium parvum, Caryospora simplex* , and *Caryospora bigenetica* can be grown from sporozoites to unsporulated/sporulated oocysts in cell cultures (Upton et al., 1984; Sundermann et al., 1988; Strout and Schmatz, 1990; Upton, 1997). In vitro culture systems offer an excellent system for examining many aspects of coccidial biology and will be essential in identifying factors involved in developmental stage conversion and induction of the sexual cycle in coccidian parasites.

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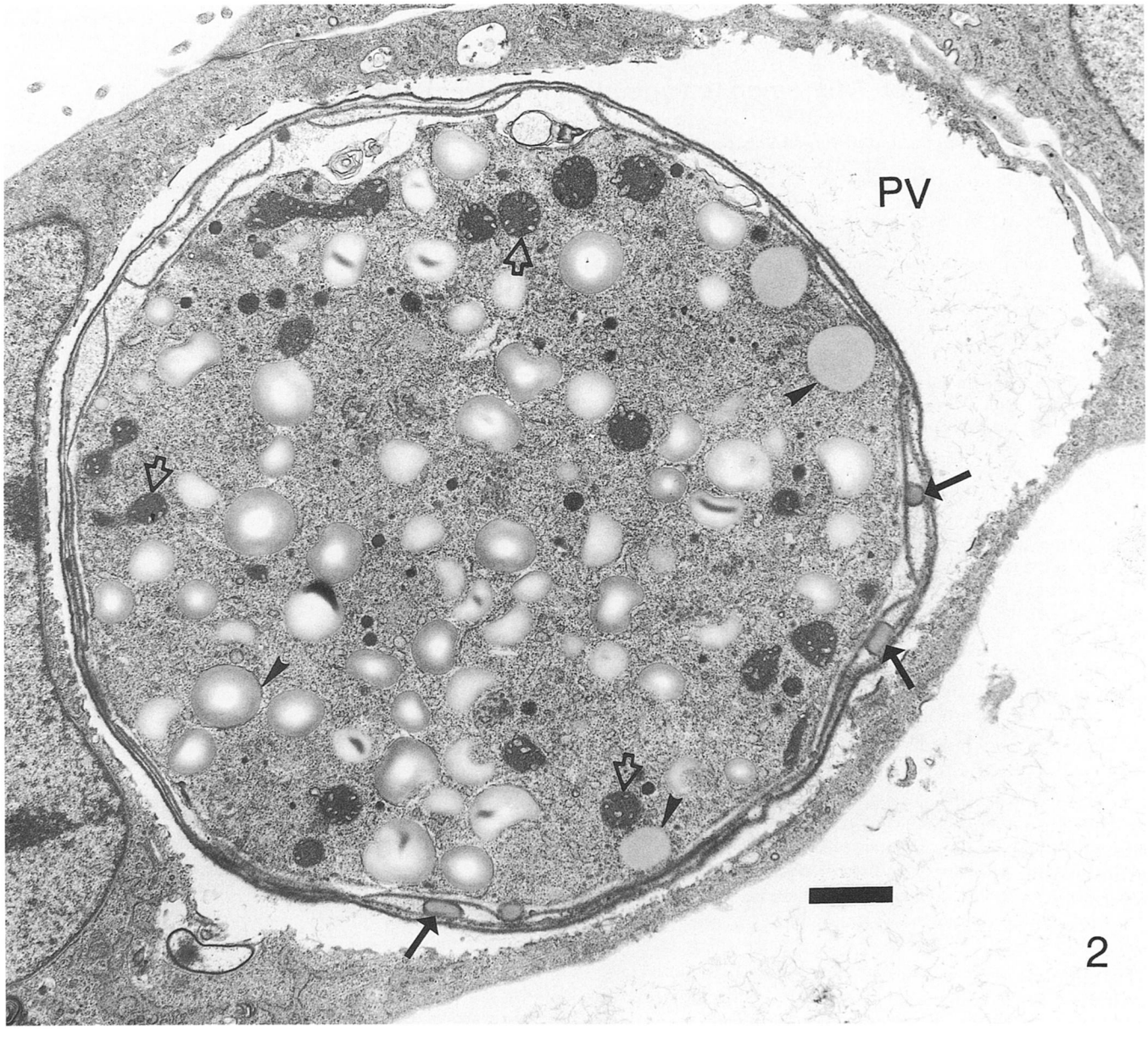

FIGURE 2. Transmission electron micrograph of a developing oocyst of *Isospora suis* in a swine testicular cell. The oocyst is in a parasitophorous vacuole (PV). Note the wall-forming bodies type I (arrowheads) and wall-forming bodies type II (open arrows). Some of the wall-forming bodies type I (arrows) have been exocytosed and are between membranes in the developing oocyst wall. Bar = 1 μm.

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