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STRUCTURES OF THE LIFE CYCLE OF *CYCLOSPORA CAYETANENSIS* (PROTOZOA, COCCIDIA) IN THE FECES OF PATIENTS WITH DIARREIC SYNDROME

ESTRUCTURAS DEL CICLO DE VIDA DE *CYCLOSPORA CAYETANENSIS* (PROTOZOA, COCCIDIA) EN LAS HECES DE PACIENTES CON SINDROME DIARREICO

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RESUMEN

La epidemiología de *Cyclospora cayetanensis* ha sido gradualmente documentada, pero su ciclo de vida no ha sido descrito completamente. Nuestros estudios en pacientes con síndrome diarreico nos han revelado no sólo la presencia de ooquistes sino esquizontes y merozoitos de tres generaciones diferentes característicos de los coccidia, los cuales se hacen presentes en las heces debido a la lisis de las vellosidades intestinales ocasionada por la presencia y desarrollo del parásito. Los esquizontes de primera generación presentan merozoitos esféricos, pudiendo ser: dizoicos, tetrazoicos, octozoicos, polizoícos. Los esquizontes de segunda generación presentan merozoitos ovales y más pequeños y los de tercera generación son los más pequeños y falcifonnes. También hemos podido detectar estructuras del ciclo sexual: gamontes indiferenciados, macrogametos, microgametocitos maduros y cigotes. Al mismo tiempo, hacemos un ligero recuento de la Cyclosporiosis en Venezuela, la cual se reportó por primera vez en nuestro país por Báez *et al.*, 1993, 1995, y desde entonces hemos intensificado la investigación en esta parasitosis desde el punto de vista clínico, epidemiológico, histopatológico y terapéutico en diferentes grupos etarios de varias comunidades, tanto en individuos inmunocompetentes como inmunocomprometidos. Se han realizado infecciones experimentales en patos y roedores de laboratorio demostrando la inespecificidad de hospedador y diseminación visceral. Hemos detectado ooquistes de *C. cayetanensis* en agua de río adyacente a comunidades estudiadas, demostrando también la presencia del parásito en cerdos, perros y gatos.

SUMMARY

Although the epidemiology of *Cyclospora cayetanensis* has been gradually documentated, its life cycle has not been completaly described. Our studies of patients with diarrheic syndrome have observed in the feces not only oocysts, but also schizonts and merozoites of three generations, characteristic of coccidians. These are released by lisis of the intestinal villi in which the parasite develops. Schizonts of the first generation contain spheroidal merozoites, wether dizoic, tetrazoic, octozoic or polyzoic. The second generation schizonts contain smaller, ovoidal merozoites, and the schizonts of the third generation contain even smaller, falciform merozoites. Sexual forms were also seen: undifferentiated gamonts, macrogametes, mature microgametocytes and zygotes. We submit here also a tentative survey of Cyclosporidiosis in Venezuela, first report by Báez *et al.*, 1993, 1995. Clinical studies were made on different age groups of various communities on both immunocompetent and immunocompromised individuals. Ducklings and laboratory rodents were inoculated with the parasite and development in their viscera observed. Oocysts were detected in the river waters adjacent to the studied communities, and also in the domestic pigs, dogs and cats.

Palabras clave: *Cyclospora cayetanensis* (Protozoa, Coocidia Humano), Ciclo vital

Keywords: *Cyclospora cayetanensis* (Human Protozoa, Coocidia), Vital Cycle

INTRODUCTION

Cyclospora cayetanensis were first observed in humans in 1979 in Papua, New Guinea (Ashford, 1979) but this organism were reported and described frequently as "*Cyanobacterium*-like bodies" because they were thought to resemble blue green algae (Long *et al.*, 1990; Hoge 1993). It was in Lima, Peru where definitively was determined their identity as a coccidian, with their characteristic oocyst disporocyst and tetrazoic: two sporocysts with two sporozoites each, observed in feces of diarrheic children (Ortega *et al.*, 1993). International descriptions report *C. cayetanensis* from North, Central and South America, the Caribbean, Africa, Asia, Australia, Europe (England, France, Spain, Italy). Taxonomically, *C. cayetanensis* has been placed in the Subkingdom Protozoa, Phylum Apicomplexa, Subclass Coccidia, Order Eucoccida, Family Eimeriidae.

In immunocompetent patients presenting Clinical Diarrheic Syndrome we have observed not only oocysts, but also other structures of stages in the life cycle of *C. cayetanensis*. These were trophozoites, schizonts with merozoites, gamonts, macrogametes, mature microgametocytes and zygotes.

Most descriptions of *C. cayetanensis* have been of mature or inmature oocysts; in some cases men-tion has been made of schizogonic forms within epithelial cells of the intestine in ultrastructural studies (Pollock *et al.*, 1992; Bendall *et al.*, 1993; Connor *et al.*, 1993; Sun *et al.*, 1996; Soave *et al.*, 1996; Ortega *et al.*, 1997).

Like all the coccidia, this species has intra-cytoplasmic life cycle, preferely within enterocytes, where it can reproduce both asexually and sexually. When, as a result of the action of the parasite, the intestinal villi are lysed, various stages of the life cycle are released into the intestinal lumen, and are carried along by the feces. Staining by the technique of Kinyoun reveals the parasites colored pale pink to intense fuchsia red, since *C. cayetanensis* is acicd resistant.

MATERIALS AND METHODS

The feces of patients were collected in plastic containers, with 2.5% Potasium Dichromate ($K_2Cr_20_7$). The containers were left uncovered at room temperature, care being taken to avoid driying. Direct microscopical observation established the presence of oocysts and of the complete maturation.

The smears were made on coverslips, allowed to dry, and fixed with methanol. After drying again, the films were covered with Kinyoun stain for 15-30 minutes, washed in tap water, differentiated with 3% hydrochloric alcohol, washed again, and counterstained 30 seconds with methylene blue, washed again and allowed to dry.

RESULTS

In the feces of patients positive for *C. cayetanensis* almost all stages in the life cycle were observed. The diarrheic syndrome of cylosporiosis, involving lysis of the intestinal villi, results in the appearence of the various forms of the parasites in the feces.

Oocysts: Inmature forms are spheroidal, with a doubled membrane, and spheroidal yolk with granulations. Mature oocysts are disporocystic and tetrazoic, each of which contains two sporozoites. The sporocysts have a Stieda body. The oocyst measure 10-11 μ. (Figs. l, 2).

Sporozoites: Falciforms, measuring 10-12 μ.

Trophozoites: Rounded, staining pale pink, measuring 2-3 μ.

Schizonts: These may be of three different generations. Those of the first generations may be dizoic, tetrazoic, octozoic or polyzoic (Figs. 3, 4, 5).

They have a thin membrane, stain fucsia, the merozoites are rounded, and measure 2.5-3 μ. On rupture of the schizonts, the released merozoites may differentiated into trophozoites or gamonts. The second generation contain smaller, and more numerous merozoites with ovoid form, measure 2 X 2.5 μ (Fig. 6, 7). The schizonts of the third generation measure 12-14 μ, and their merozoites have lost the rounded form and they are some falciforms and smaller, measuring 1.5 X 2 μ (Fig. 8).

Gamonts: Rounded, staining red, measuring 5-7 μ.

Gametes: Mature microgametocytes are spheroidal (7-8 μ), Containing numerous microgametes, in form resembling a comma, measuring 1 X 1.5 μ each, and stain black. Characteristic of the

mature microgametocyte is an excentric residual mass among the microgametes (Fig. 9). The female macrogametocyte has a nucleus and numerous reserve granules that move toward the membrane, coming to lie at the internal face of the membrane at complete maturity they measure 8.5 - 9 μ (Fig. 10).

Zygote: This is spheroidal, with a central nucleus and an excentric vacuole. It measures 9 - 9.5 μ.

DISCUSSION

The epidemiology of *Cyclospora cayetanensis* has been gradually documented, but its life cycle has not be completely described; up to the present, only its role as an intracellular intestinal protozoan has been considered as a typical coccidian.

Endoscopy has revealed the schizogonic cycle of *C. cayetanensis*, with marked erythema in the distal part of the duodenum. Duodenal aspirates have showed atrophy of the villi and hyperplasia of the crypts; ultrastructural studies have described schizonts and merozoites in vacuoles of the epithelial cells of the jejunum (Pollock *et al.*, 1992; Bendall *et al.*, 1993; Connor *et al.*, 1993; Sun *et al.*; 1996; Deluol *et al.*, 1996; Soave *et al.*, 1996; Ortega *et al.*,1997).

Bendall *et al.*, 1993 in a jejunal biopsy from a patient showing diarrhea, weight loss, nausea and anorexia, observed intracellular parasites within a vacuole.

Connor *et al.*, 1993 found inflammatory changes, atrophy of villi, and hyperplasia of the crypts in patients with diarrheic feces that were positive for *C. cayetanensis*.

Sun *et al.*, 1996, by light and, ultramicroscopy identified *C. cayetanensis* in the human small intestine, finding there schizonts, sporozoites and trophozoites, with oocysts occurring only in the feces.

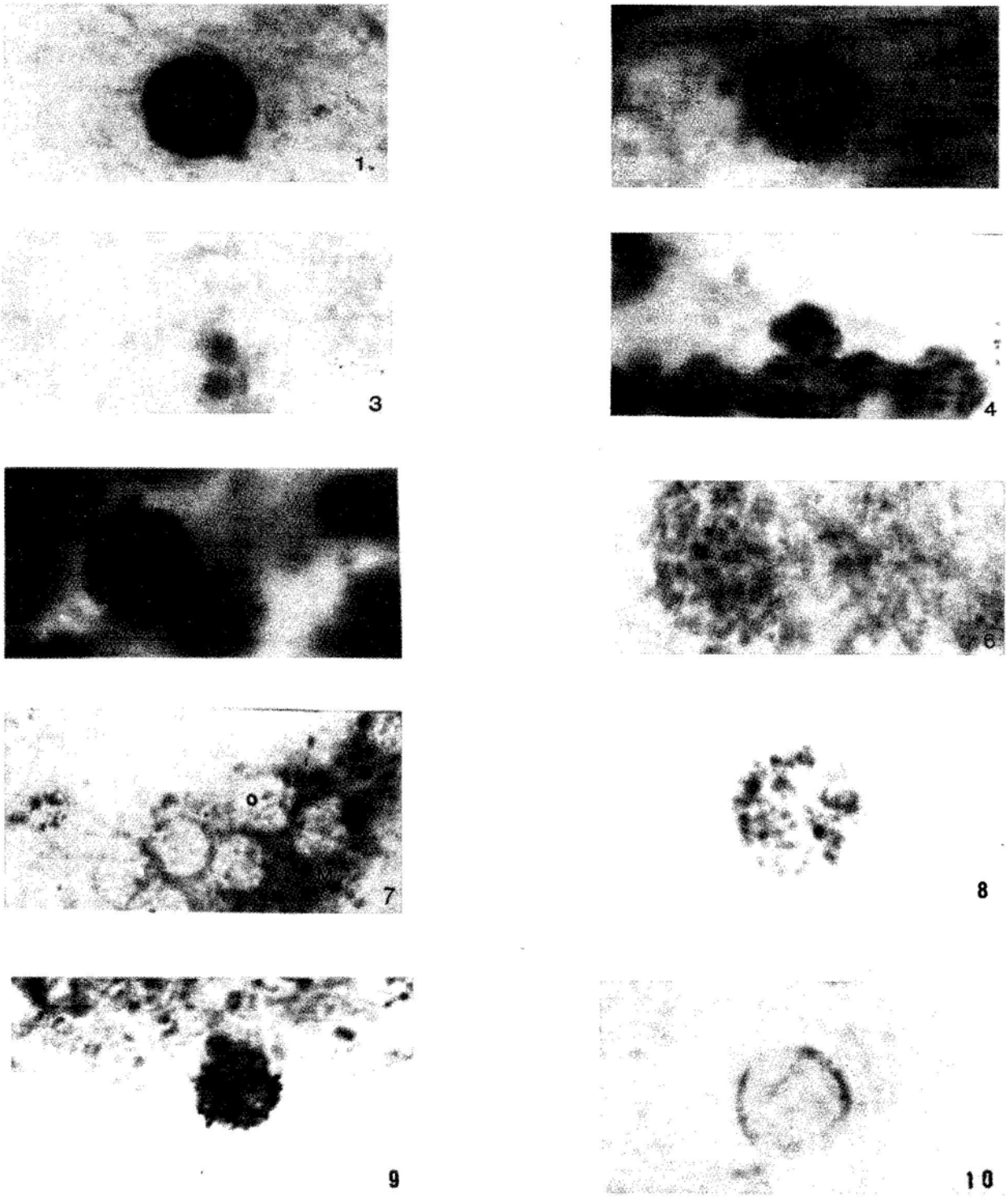
Deluol *et al.*, 1996, reporting the first four cases of *Cyclospora* in France, in a routine biopsy from a patient for histological study by light and electron microscopy, observed intracytoplasmic vacuoles measuring 6-8 μ within the surface of the enterocytes. These structures were identified as merozoites enveloped in the parasitophoric vacuole; they were ovoid in longitudinal section and polygonal transversely. They were highly polarized, with nuclei containing a prominent nucleolus, micronemas at one extremity and roptrias at the other. The observed alterations varied from light to moderate, with inflammatory infiltration of leucocytes, polymorphonuclears, lymphocytes, plasmatic cells and eosinophils in the lamina propia, hyperplasia of the crypts, lengthening of the crypts characterized by the shortening of the villi also the enterocytes tent to become columnar or cuboidal.

Ortega *et al.*, 1997 describes meronts with merozoites and a sexual form represented by a spherical structure with reserve granules, which resemble a macrogametocyte in differentiation because the reserve granules observed still are not ubicated adossed to internal part of the membrane, which is the characteristic of a mature macrogametocyte in the coccidia.

Our studies in immunocompetent patients with diarreic syndrome and oocysts of *C. cayetanensis* have revealed three different generations of merozoites, both within and outside the schizonts (meronts), typical of coccidia these forms being present in the feces due to lysis of the villi containing the parasites.

Thus, schizonts of the first generation, wether dizoic, tetrazoic, octozoic or polyzoic, contain spheroidal merozoites (Figs. 3, 4, 5, 9), The schizonts of the second generations contain smaller, oval merozoites (Fig. 6, 7), and schizonts of the third generation contain even smaller falciform merozoites (Fig. 8). Also, in the feces, sexual, stages undifferentiated gamonts that are larger tham first generation merozoites, macrogametes, microgametocytes and zygotes. (Figs. 9, 10).

The first case of cyclosporidiosis in Venezuela was réported by Báez *et al.*, 1993, 1995a. Since then we havé sought and studied *C. cayetanensis* clinically, epidemiologically and experimentally with histiopathology.



Figures 1-10. Structures of the life cycle of *Cyclospora cayetanensis* in feces of patients. 1. Mature oocyst; 2. Mature oocyst (see the two sporocysts); 3. Dyzoic schyzont (1st. generation); 4. Tetrazoic schizont (1st generation); 5. Polyzoic schizont (1st. generation); 6. Schizont (2nd generation) with ovals merozoites; 7. Free merosoites (2nd generation) (M) and empty oocysts (o); 8 Schizont (3rd. generation) with falciform merozoites; 9. Meture mycrogametocyte with mycrogametes of black color and the characteristic residual mass of the mycrogametocyte; 10. Mature macrogamete with the characteristic reserve granules adossed to internal side of the cellular membrane

SCHEME I

Ooocyst:	10-11 µ		
Sporozoites:	10-12 µ		
Schizonts	1st generation	Dizoics	3 µ
		Tetrazoics	2.5-3 µ
		Octozoics	2.5-3 µ
		Polyzoics	2.5-3 µ
	2nd generation		2-2.5 µ
	3rd generation		1.5-2 µ
Trophozoites			2-3 µ
Gamonts			5-7 µ
Microgametocyte			7-8 m
Microgamets			1x1.5 µ
Macrogamets			8.5-9 µ
Zigote			9-9.5 µ

Clinically, we have studied different age groups in various communities, both in immunocompetent and immunocompromised individuals, these latter being immunodeficient (HIV^+) and immunosuppresed (Báez *et al.*, 1995a,b,c; Báez *et al.*, 1998a,b,c; Campo-Aasen and Báez 1994; Morales *et al.*, 1998; Salazar *et al.*, 1994; Reverand *et al.*, 1998; Arcay *et al.*, 1998; Baez *et al.*, 2000).

From feces of immunocompetent patients, we have recognized the stages in the life cycle of *C. cayetanensis*, as described previously (Arcay *et al.*, 1995, 1996, 1997, 1998; Arcay and Bruzual, 1995; Bruzual and Arcay 1997), now here presented. At the same time we propose a schematic life cycle in accord with its status as Coccidia, Eimeriidae (Scheme II).

The immunocompetent patients, of both sexes and from different age groups and communities, showed diarreic syndrome with explosive evacuations, seldom with mucus, without blood, accompanied by anorexia, nausea, abdominal cramps, flatulent dispepsia, weight loss and malabsorption.

Experimentally, ducklings two months of age were inoculated per os with *C. cayetanensis* from an immunocompetent patient. Dr. Zerpa, in Peru (1995) had found the parasite in the feces of a duck belonging to a family with three members parasitized. The ducklings experimentally parasitized in our laboratory (Arcay and Bruzual, 1995) produced diarreic feces and showed visceral dissemination of the parasite, not only in the digestive tract, but in the liver, gall bladder, spleen, kidney and adrenal glands. There was visible pathogenesis in these organs, as well as the stages in the life cycle of *C. cayetanensis*: inmature oocysts, meronts, macrogametes and mature microgametocytes. In the same manner, laboratory rodents were infected, producing a similar histopathological picture in the majority of the viscera, and the appearance of *C. cayetanensis* stages (Arcay *et al.*, 1997; Bruzual and Arcay, 1997).

Investigating the presence of the three enteropathogens in river waters in relation to AIDS, we detected *C. cayetanensis* in concomittance with *Cryptosporidium parvum* and *Microspora* in the river water and in the immunocompetent individuals of the two populations of both river banks. (Arcay *et al.*, 1998) Oocysts of *C. cayetanensis* were inoculated into NMRI mice, from which parasite were detected in feces and urine, not only in the initial months post-infection, but up to a year later, in one mouse.

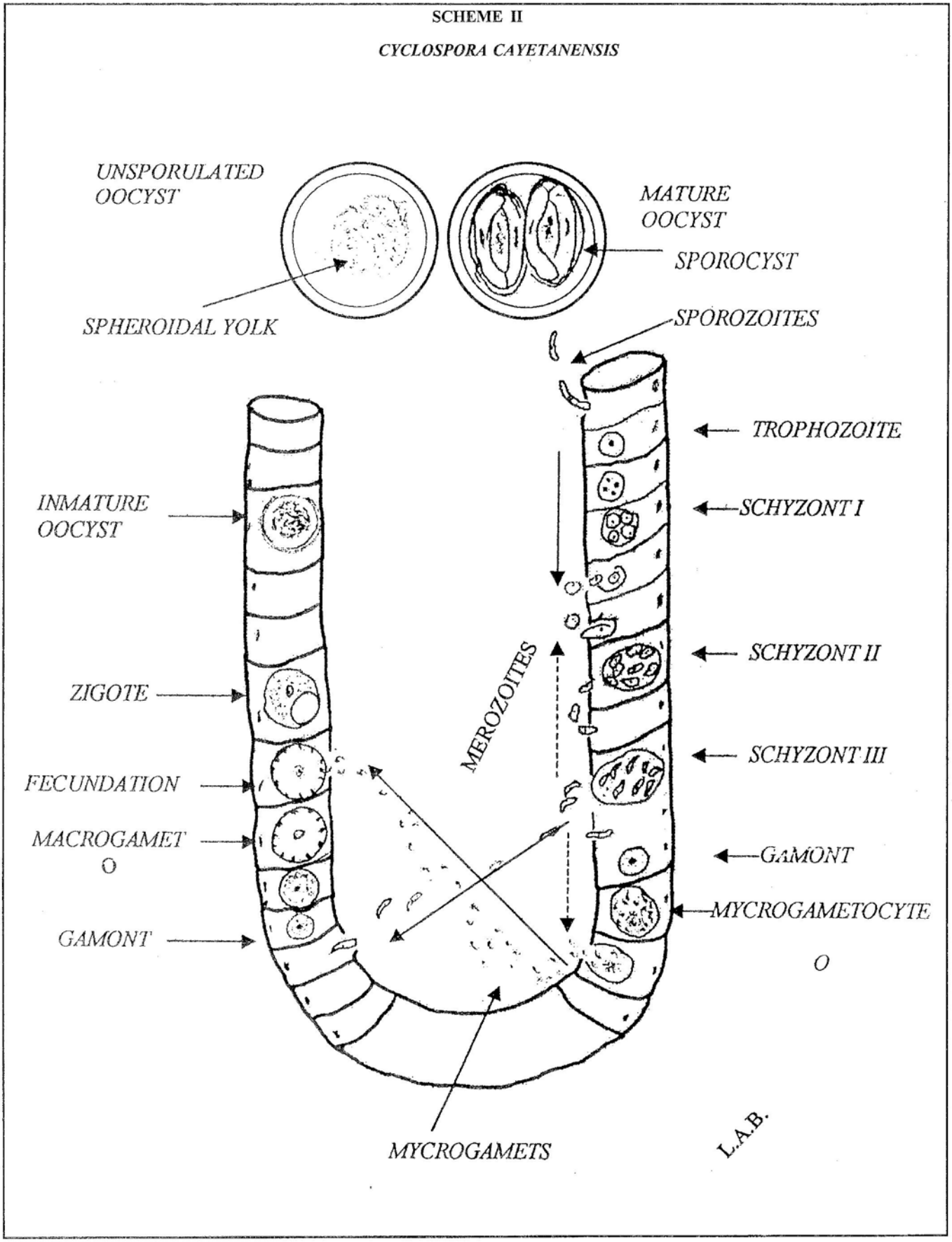
We consider cyclosporidiosis to be a zoonosis, since *C. cayetanensis* we have detected in the pigs, dogs and cats of the river communities of that study (Arcay *et al.*, 1998).

Nevertheless in our laboratory we have demonstrated in ducks, mice, hamsters and rats, infected experimentally with *C. cayetanensis* from feces of immunocompetens patients the visceralization of *C. cayetanensis*, observing not only in the digestive tract in the histopathological study, but also in liver, gallbladder, kidney, spleen and suprarenal glands, where we have observed asexuals and sexuals structures of the life cycle of C. cayetanensis. (Arcay and Bruzual, 1995; Arcay *et al.*, 1997; Bruzual and Arcay, 1997).

About the inspecificity of *C. cayetanensis* in other animal hosts, Sterling and Ortega, 1999 considers that the Cyclospora described from ducks, chickens and not human primates (Zerpa *et al.*, 1995; Garcia-López *et al.*, 1996 Smith *et al.*, 1996; Yai *et al.*, 1997), be the same *C. cayetanensis*.

SCHEME II

CYCLOSPORA CAYETANENSIS



Recently *C. cayetanensis* has been found in vegetable foods eaten without cooking (lettuce, watercreess, cabbage, coriander, strawberries and blackberries) incertain communities (Rios *et al.*, 1999; Castellano *et al.*, 1999). Also Rios *et al.*, (o.c) have detected *C. cayetanensis* by the polymerase chain reaction (PCR).

REFERENCES

ARCAY, L., E. BAEZ DE BORGES AND E. BRUZUAL

1995. Estructuras características del ciclo sexual y asexual de *Cyclospora cayetanensis* (Coccidia, Eimeriidae) en pacientes con síndrome diarreico.XII Congreso Latinoamericano de Parasitología (FLAP), Santiago de Chile. *Parasitología al Día*, Chile, 19, pag. 277.

ARCAY, L., E. BAEZ DE BORGES AND E. BRUZUAL

1996. Epidemiology and biology of *Cyclospora cayetanensis* Joint Meeting of the American SOC. Protozoologists.. Meeting Opportunistics Protozoa, Tucson, Arizona.

ARCAY, L., E. BRUZUAL AND E. BAEZ DE BORGES

1997. Diseminación de *Cyclospora cayetanensis* en roedores de laboratorio. Histopatología.XIII Congreso de la Federación Latinoamericana de Parásitólogos (FLAP), La Habana, Cuba (FLAP)

ARCAY, L., E. BAEZ DE BORGES AND E. BRUZUAL

1998. Incidencia de *Cyclospora cayetanensis* y *Microspora* en pacientes de la población circunvecina del río Anare (Venezuela) y su presencia en el agua del rio.*Archivos del Hospital Vargas,* Caracas, 40 (3-4): 157-162.

ARCAY, L. AND E. BRUZUAL

1995. El pato doméstico (*Anser domesticus*) como hospedador experimental de *Cyclospora cayetanensis* Ortega *et al.*, 1993 (Coccidia, Eimeriidae)XII Congreso Latín. de FLAP, Santiago de Chile.- *Parasitología al Día*, Chile, 19, pág. 337.

ASHFORD, R. W.

1979. Occurrence of an undescribed coccidian in man Papua New Guinea. *Annals Trop. Med.Parasitology*, 73: 497-500.

BAEZ DE BORGES, E., G. URQUIOLA, M. URRESTARAZU, I.CAMPO-AASEN, N. SERRANO, Z. CARVAJAL AND Y. ASCANIO

1993. Etiopatogenia de las diarreas infecciosas crónicas en el adulto.XI Congreso de FLAP - I Congreso Peruano de Parasitología, Núm. 41, pág. 88.

BAEZ DE BORGES, E.

1995a. Primer caso de Cyclosporiosis reportado en Venezuela. *Rev. Federación Médica Venezolana*, 6(1): 211-215.

BAEZ DE BORGES, E., C. SCHNEIDER AND D. MORON

1995b. Ascítis Con peritonitis granulomatosa en la infecciòn intestinal por *Cyclospora cayetanensis. Archivos Hospital Vargas,* 37 (1-2):47.

BAEZ DE BORGES, E., L. ARCAY AND G. DUQUE

1995c. Uso de Albendazol y Trimetoprim-Sulfametoxazol en Cyclosporiosis y Microsporidiosis experimental. *Parasitología al Día*, Chile, 19, pág. 337.

BAEZ DE BORGES, E., L. ARCAY, AND G. DUQUE

1998a. *Cyclospora cayetanensis* y *Microspora* en las heces de pacientes con SIDA y síndrome diarreico. *Rev. Federación Médica Venezolana*, 6 (1) Sección II.89-96

BAEZ DE BORGES, E., L. ARCAY AND G. DUQUE

1998b. Uso de Albendazol y Trimetoprim-Sulfametoxazol en Cyclosporiosis y Microsporidiosis. *Rev. Federación Médica Venezolana.*, 6 (II) Sección II. 28-34

BAEZ DE BORGES, E., L. ARCAY, S. REVERAND AND E. OTERO

2000. Microspora: Ethiological agents in chronic diarrhoea. *Bol. Soc. Venez. Microbiol.* 20(1): 53-56.

BENDAL, R. P., S. LUCAS, A. MOODY, G. TOVEY AND P.L. CHIODIN

1993. Diarrhoea associated with *Cyanobacterium*-like bodies: a new coccidian enteritis of man. *The Lancet* 341: 590-592.

BRUZUAL, E. AND L. ARCAY

1997. *Cyclospora cayetanensis* y Microspora en tejidos glandulares.XII Cong. Latinoamer. Parasitologia (FLAP), La Habana, Cuba.

CAMPO-AASEN, I. AND E. BAEZ DE BORGES

1994. *Cyclospora cayetanensis* y otros enteropatógenos en estudiantes de Medicina en Venezuela. Intern, Cong. Parasitology (ICOPA VIII). Turquía. 2:23.

CASTELLANO, A., L. BOTERO, L. ROMERO AND L. ARCAY

2000. Detección de Protozoarios parásitos en vegetales de granjas y mercados de los Municipios Maracaibo y San Francisco del Edo. Zulia (Venezuela). II Congreso Venez. Microbiología, Maracaibo, p. 116.

CONNOR, B. A., D.R. SCHLIM, J.V. SCHOLES, J.L. RAYBURN, J. REIDY AND R. RAJAH

1993. Pathologic changes in the small bowel in nine patients with diarrhoea associated with a coccidia-like body. *Ann. Intern. Med.*, 119: 377-382.

DELOUL, A. M., M.F. TEILHAC, J.L. POIROT, F. HEYER, L. BEAUGERIEL AND F.P. CHATELET

1996. *Cyclospora* sp. Life Cycle Studies in patient by Electron-Microscopy. *J. Euk. Microbiology*, 43(5) Supp.: 1285-1295.

HERWALDT, B.L., M.L. ACKERS AND CYCLOSPORA WORKING GROUP.

1997. An outbreak in 1996 of *Cyclospora* associated with imported raspberries. *N. Engl. Med.*, 336: 1548-1556.

HOGE, C.W., D.R. SCHLIM, R. RAJAH, J. TRIPLETT, M. SHEAR, J.G. RABOLD AND P. ECHEVERRIA

1993. Epidemiology of diarrhoeal illness associated with coccidian-like organism among travellers and foreign residents in Nepal. *Lancet* (British edition) 34 (8854).

LONG, E.G., A. EBRAHIMZADEH, E.H. WHITE, B. SWISHER AND C.S. CALAWAY

1990. Alga associated with acquired immunodeficiency syndrome and in travelers. *J. CIin. Microbiol.*, 28: 1101-1104.

MORALES, J., E. BAEZ DE BORGES, J. MA.RTIN, E. OTERO, G. RODRIGUEZ, ET AL.,

1994. Microsporidia y otros enteropatógenos (*Cyclospora cayetanensis*) en pacientes con SIDA. *Boletín Venezolano Infectología*, 4 (3-4), pág. 20.

ORTEGA, I., CH.R. STERLING, R.H. GILMAN, V.A. GAMA AND F. DIAZ

1993. *Cyclospora* species- A New Protozoan pathogen of humans. *The New England j. Med.*, 328, (18): 1308-1312.

ORTEGA, Y. R., C.R. ROXAS, R.H. GILMAN, N. MILLER, L. CABRERA, C. TAQUIRE AND CH.C. STERLING

1997. Isolation of *Cryptosporidium parvum* and *Cyclospora cayetanensis* from vegetables collected in markets of an endemíc regíon in Perú. *Am. J. Trop.Med. Hyg.*, 57: 683-686.

ORTEGA, Y, R. NAGLE, R. GILMA, J. WATANABE, J. MIYAGI, H. QUISPE, P. KANAGUANKA, C. REXOS AND CH. STERLING

1997. Pathologic and Clinical findings in patients with Cyclosporiasis and a description of intracellular parasite. Life cycle stages. *J. Inf. Dis.*, 176: 1584-1589.

POLLOK, R.C.G., R.P.BENDALL, A. MOODY, P.L. CHIODINE AND D.R. CHURCHIL

1992. Traveler's diarrhoea associated with cyanobacterium-like bodíes (letter). *Lancet*, 340: 556-557.

REVERAND, S., E. BAEZ DE BORGES AND L. ARCAY

1999. *Cyclospora cayetanensis* en niños asintomáticos del área metropolitana de Caracas. *Rev. Feder. Méd. Venezolana.* 7(1): 26-31.

RIOS DE SELGRAD, M. AND M.L. NOVOA

1999 Evaluación de la calidad higiénica e incidencia de parásitos entéricos en las vegetales crudos que se consumen en Caracas. XIV Congt. Latinoam. Parasitol. (FLAP), Acapulco, México, p. 27.

SALAZAR, W. AND KS. ROJAS

1999. Cyclosporiosis intestinal sintomática en adulto venezolano. *Acta Científica Venezolana* 45(1), pág. 249

SMITH, H. C. PATON, R. GIRDWOOD AND M MTAMBO

1996. *Cyclospora* in non-human primates in Gombe, Tansania.. *Vet. Rec.*, 138:528.

STERLING, CH., AND Y. ORTEGA

1999. *Cyclospora*: An enigma worth unraveling. *Emer. Inf. Des.*, 5(1):48-53.

SOAVE, R.

1996. *Cyclospora* : an overview. *Clinical Infect. Diseases*, 23(3):429-437.

SUN, T., C.F. ILARDI, A.R. BRESCIANI, S. GOLDENBERG, B. ROBERTS AND KS. TEICHBERG

1996. Light and electron microscopic identification of species in the small intestine: evidence of the presence of asexual life cycle in human host. *Ann. Clinical Pathol.*, 105(2): 216-220.

YAI, L., A. BAUA, M. HIRSCHFELD, M. DE OLIVEIRA, J. DAMACEN

1997. The first tow cases of *Cyclospora* in dogs (Sao Paulo, Brasil). *Rev. Inst. Med. Trop.*, Sao Paulo, 39: 177-179.

ZERPA, N. UCHIMA AND L. HUICHO

1995. *Cyclospora cayetanensis* asociated with watery diarrhoea in Peruvian patients. *J. Trop. Med. Hyg.*, (England) 98(5): 352-329.