HUMAN MICROSPORIDIOSES AND AIDS: RECENT ADVANCES

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Summary :

Microsporidia, unicellular parasites frequent in animals, were rarely reported in humans before the advent of AIDS. This immunodeficiency syndrome induces the emergence or resurgence of opportunistic infections such as microsporidiosis. Since 1985, five species of microsporidia have been found in HIV-infected patients. One of these species was already known in animals whereas all others are new. An increasing number of cases of microsporidiosis is detected due to the improvement of methods of diagnosis. According to a study conducted in USA, intestinal microsporidia appear to be the first cause of diarrhea in patients with AIDS. These parasites are intensively investigated as shown by the increasing number of studies published since 1993. Most data concern the diagnosis, pathology, therapy and epidemiology of human microsporidiosis as well as the characterization of their agents. Experimental studies aiming to define the immune context of these infections are also reported.

KEY WORDS : microsporidia, microsporidiosis, diagnosis, pathology, therapy, SIDA.

Résumé : MICROSPORIDIOSES HUMAINES ET SIDA

Les microsporidies, parasites unicellulaires fréquents chez les animaux, étaient peu connues chez l'homme jusqu'à l'apparition du SIDA. Ce syndrome entraîne la résurgence ou l'émergence d'infections opportunistes parmi lesquelles figurent les microsporidioses. Cinq espèces de microsporidies dont quatre nouvelles ont été trouvées chez les patients atteints de SIDA et, avec l'amélioration des méthodes de diagnostic, le nombre des cas rapportés de microsporidioses ne cesse d'augmenter. Les atteintes digestives sont les plus fréquentes et, selon une enquête récemment effectuée aux États-Unis, les microsporidies intestinales seraient la première cause de diarrhées chez les patients atteints de SIDA. Ces parasites font l'objet d'un effort d'investigation intense comme le montre la quantité d'études publiées depuis 1994. Les informations ici rapportées sont relatives au diagnostic, aux aspects cliniques, thérapeutiques et épidémiologiques des microsporidioses ainsi qu'à la caractérisation de leurs agents. L'approche expérimentale visant à définir le contexte immunitaire de ces infections est également évoquée.

MOTS CLÉS : microsporidies, diagnostic, pathologie, thérapie, SIDA.

INTRODUCTION

Microsporidia are unicellular parasites which infect a wide range of invertebrates including protozoa such as ciliates (Foissner & Foissner, 1995). Also known in fish, birds and mammals (Canning & Lom, 1986), they were rarely reported in humans before the advent of AIDS. Since 1985, four new species of microsporidia have been found in AIDS patients (Table I) and these parasites are gaining increasing attention as opportunistic pathogens responsible for significant morbidity. Most recent data, concerning human microsporidiosis and their agents, are herein reported.

GENERAL CHARACTERS OF MICROSPORIDIA

These protists are considered to be ancient eukaryotes. They have the nuclear organization of eukaryotes but they lack mitochondria, per-

Microsporidia	Clinical symptoms
<i>Enterocytozoon bieneusi</i>	Abdominal cramping, nausea, weight loss, malabsorption, zinc deficiency, chronic diarrhoea, cholecystitis, cholangitis, rhinosinusitis
<i>Encephalitozoon</i> (= <i>Septata</i>) <i>intestinalis</i>	Diarrhoea, nausea, malabsorption, weight loss, fever, cholecystitis, rhinosinusitis, bronchiolitis, nephritis
<i>Encephalitozoon hellem</i>	Keratoconjunctivitis, sinusitis, bronchiolitis, nephritis, ureteritis, dysuria, prostatitis
<i>Encephalitozoon cuniculi</i>	Dysuria, nephritis, sinusitis, bronchitis, hepatitis, peritonitis
<i>Trachipleistophora hominis</i>	Myositis
<i>Nosema connori</i>	Disseminated infection
<i>Nosema ocularium</i>	Corneal stroma infection
<i>Vittaforma corneae</i> (= <i>Nosema corneum</i>)	Corneal stroma infection

Table I. – Microsporidian species parasite of man and their clinical manifestations according to the literature cited in text. *N. ocularium* Cali *et al.*, 1991 and *V. corneae* Silveira & Canning, 1995 have been only found in HIV-negative patients. The unique case of infection by *N. connori* Sprague 1974, has been reported in an athymic infant. *E. cuniculi* is also parasitic in animals (carnivora, lagomorphs, rodents).

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oxysomes, Golgi membranes, and their ribosomal RNA sequence is similar to that of prokaryotes (Vossbrinck *et al.*, 1987). Their development occurs within cells of their hosts. It consists of a division phase followed by a spore producing phase. The only extracellular stages

are mature spores which are released in the extracellular medium after the host cell death. Microsporidian spores possess a typical polar tube of which extrusion ensures the inoculation of the infecting sporoplasm into a host cell (Fig. 1).

Fig. 1. – Transmission electron micrograph showing three spores of *Enterocytozoon bienewsi* at different stages of maturation in the cytoplasm of an enterocyte. Sections of the polar tube (arrowheads) are seen in the sporoplasm. These spores measure approximately $1.5 \times 0.8 \mu\text{m}$. Scale bar = $0.5 \mu\text{m}$.

AIDS RELATED MICROSPORIDIOSES

Clinical manifestations of microsporidiosis depend on the site of the infection. *Enterocytozoon bieneusi*, (Desportes *et al.*, 1985), develops in the enterocytes of the small intestine. The species causes chronic diarrhea, abdominal cramping and nausea; it is also responsible for the malabsorption and the severe weight loss reported in AIDS patients, and appears to be involved in zinc deficiency as shown by the low levels measured in patients (Asmuth *et al.*, 1994). This microsporidian could thus contribute to the progression to AIDS since deficiency in zinc is known to be related with progression to this syndrome (Graham *et al.*, 1990 cited in Asmuth *et al.*, 1994). The parasite has also been found in HIV infected patients without chronic diarrhea (Rabeneck *et al.*, 1995). Although small intestine is the primary site of infection, *Ent. bieneusi* can also spread to the biliary ducts and nasopharyngeal epithelium, causing thereby some cases of cholangitis and rhinosinusitis (Weber *et al.*, 1994). *Encephalitozoon* (= *Septata*) *intestinalis* is the second species known to cause diarrhea in AIDS patients (Cali *et al.*, 1993; Hartskeerl *et al.*, 1995). *Encephalitozoon bellem* has been isolated from ocular biopsies of patients with keratoconjunctivitis (Didier *et al.*, 1991). Whereas *Ent. Bieneusi* appears to be restricted to epithelia, all species of the genus *Encephalitozoon* develop in a variety of cells including macrophages thus causing disseminated infections. *Encephalitozoon intestinalis*, *Enc. bellem* and *Enc. cuniculi*, a species already known to infect mammals, can cause rhinosinusitis, bronchiolitis, nephritis, ureteritis, hepatitis as well as peritonitis (Cali *et al.*, 1993; Weber *et al.*, 1994; Gunnarsson *et al.*, 1995; Sobottka *et al.*, 1995; Didier *et al.*, 1996). Three cases of myositis were imputed to *Pleistophora* sp. (Ledford *et al.*, 1985; Chupp *et al.*, 1992; Hollister *et al.*, 1995). Peculiarities observed in the sporogony of this parasite have led to the creation of the genus *Trachipleistophora* with the species *T. hominis* (Hollister *et al.*, 1995). Of all these species, *Ent. bieneusi* is the most frequent in AIDS patients. It is reported in 14 to 40 % of HIV-infected patients with weight loss and chronic diarrhea (Asmuth *et al.*, 1994; Weber *et al.*, 1994). A recent evaluation of the prevalence of enteric pathogens in HIV-infected patients has shown that microsporidia are the major cause of diarrhea and other gastrointestinal manifestations (Kotler, 1995).

The interest of biologists and clinicians is mainly focused on the diagnosis and treatment of human microsporidiosis. Most information concerning the characterization of these pathogens and their identification is provided by the study of *in vitro* models of microsporidial infections.

TAXONOMICAL DATA

The comparison of isolates of *Encephalitozoon* (= *septata*) *intestinalis* obtained from cultures on RK13 cell line (Van Gool *et al.*, 1994) with those of *Encephalitozoon cuniculi* and *Enc. bellem* was performed by SDS-PAGE, western blotting and DNA analysis (Hartskeerl *et al.*, 1995). Due to the homology (90 %) found between gene sequences encoding for small subunits of ribosomal RNA, the species previously described under the generic name of *Septata* has been reclassified in the genus *Encephalitozoon*.

Genomic analyses have also shown that the microsporidian isolated from the urine and sputum of an AIDS patient corresponded to *Encephalitozoon cuniculi*, a species already known to infect carnivora, rodents or lagomorphs (De Groote *et al.*, 1995). Similar results were obtained by Hollister. Interestingly the human isolate differed from canine and murine isolates (Hollister *et al.*, 1995). A rabbit isolate has been also recently characterized (Didier *et al.*, 1995).

DETECTION

The identification of intracellular stages of microsporidia requires histological studies of samples obtained by invasive procedures. Therefore, the detection of the spores released in excreta such as faeces, urine, bile, duodenal, bronchial or nasal fluids appears to be the most practical method of diagnosis. However it is hampered by the small-size of microsporidian spores. Actually, the uneasy diagnosis of microsporidia has been a cause of underestimation of the number of infected patients. Electron microscopy remains the best method for the specific identification of developmental stages and also for confirming the diagnosis performed with other techniques such as the staining by the fluorochrome Uvitex 2B (Van Gool *et al.*, 1993) and the trichrome of Weber *et al.* (1992) modified by Kokoskin *et al.* (1994) which are extensively used for the identification of microsporidia in stools, urine and any other sample (De Girolami *et al.*, 1995). New specific diagnostic tools have been proposed using immunological characterization or the PCR amplification of genes coding for conserved ribosomal RNA subunits.

IMMUNODIAGNOSIS

The production of specific antibodies directed against microsporidian species as well as the detection of antibodies in patients' sera require large quantities of purified antigens. *In vitro* cultures of *Encephalitozoon*

bellem and *Enc. cuniculi* provide millions of spores which are released in the supernatants. This material has been used for immunological identification and characterization (Aldras *et al.*, 1994; Didier *et al.*, 1991, 1995; Hollister *et al.*, 1995; Schwartz *et al.*, 1993; Visvesvara *et al.*, 1995). Fluorescent antibody staining enabled the distinction between the two morphologically similar species *Enc. bellem* and *Enc. cuniculi* (Schwartz *et al.*, 1993). In the absence of experimental models of *Ent. bienewsi*, other microsporidia have been tested for their serologic cross-reactivity with this species. Both *Ent. bienewsi* and *Enc. intestinalis* were thus aspecifically detected by indirect immunofluorescent antibody tests using murine polyclonal antisera raised in BALB/c mice immunized with spores of *Enc. bellem* or *Enc. cuniculi* (Aldras *et al.*, 1994; Zierdt *et al.*, 1993). A western blot technique using *Glugea atherinae*, a microsporidian parasitic in fish, has shown the cross-reactivity of sera from patients with microsporidiosis to *Glugea* antigens (Ombrouck *et al.*, 1995). Since 1994, *in vitro* cultures of *Enc. intestinalis* permits the development of diagnostic procedures (Doultree *et al.*, 1995; Hartskeerl *et al.*, 1995). Polyclonal antibodies selectively directed against this species have been raised in rabbits (Aldras *et al.*, 1994; Visvesvara *et al.*, 1995) and mice (Hartskeerl *et al.*, 1995). Ombrouck has recently proposed a method combining the non specific labeling of microsporidian spores by the fluorochrome Uvitex 2B and an indirect immunofluorescent assay using a polyclonal antibody specifically directed against *Enc. intestinalis* (Ombrouck *et al.*, 1996). Additionally this method enables the distinction between spores of this species and those of *Enterocytozoon bienewsi* which are only stained by fluorochrome.

PCR DETECTION

The genomic amplification by the polymerase chain reaction for the detection and characterization of microsporidian infections in humans has been first investigated in the Netherlands and in the USA (Shuitema *et al.*, 1993; Weiss *et al.*, 1993; Zhu *et al.*, 1994). Primers are selected in the conserved DNA sequence coding for the small subunit ribosomal RNA which contains species-specific sequences and has a characteristic small size. Using these primers, (SSU)rRNA gene fragments of *Ent. bienewsi* were detected in biopsies after amplification by the PCR method. Fedorko *et al.* (1995) have proposed a PCR assay for the detection of microsporidia in stool specimens. Presently, the species identification of *Ent. bienewsi* and *Enc. intestinalis* in stool samples can be performed within some hours due to a recent improvement of the PCR method (Ombrouck *et al.*, 1996).

HOST IMMUNITY AND MICROSPORIDIAL INFECTIONS

Clinical manifestations of microsporidiosis as those of other opportunistic infections correlate with the intensity of infection and the decrease in the number of CD4⁺ cells (CD4⁺ cell counts < 50 per mm³) (Kotler, 1995). Animals models are helpful for understanding human infections and studying the relationships between microsporidia and the host immune system. A model of human encephalitozoonosis (*Enc. cuniculi*) has been obtained using SCID mice (Hermanek *et al.*, 1993; Koudela *et al.*, 1993). Experimental infections by *Enc. cuniculi*, *Enc. bellem* and *Vittaforma corneae* (= *Nosema corneum*) were also obtained by Didier *et al.* (1994) in different murine strains and monkeys. All these microsporidia cause asymptomatic and chronic infection in immunologically competent animals whereas immunocompromised ones die more or less rapidly according to the infecting species. Lethality was observed in generalized infections caused by the dissemination of the parasites by the macrophages. Experimental infections of BALB/c mice have shown that the destruction of *Enc. cuniculi* by macrophages occurred in the presence of factors released by T-lymphocytes (Schmidt & Shadduck, 1984 cited in Didier *et al.*, 1994, 1995). *In vitro* studies have demonstrated that murine macrophages could kill *Ent. cuniculi* when they were treated with IFN-gamma in combination with lipopolysaccharide; a microbiostatic activity was obtained when macrophages were treated with IFN-gamma only (Didier & Shadduck, 1994). According to Didier (1995), microbicidal or microbiostatic activities of activated macrophages occur through the nitric oxide-dependent mechanism also involved in the killing of *Leishmania major* and other pathogens (Vouldoukis *et al.*, manuscript in press). These *in vitro* data suggest that the lack of activating factors such as IFN-gamma due to TH1-cell depletion (Del Prete & Romagani, 1994) is involved in the spreading of Encephalitozoonosis in athymic mice.

TREATMENT

Clinical assays confirmed the selective eradication of all *Encephalitozoon* species by albendazole. *In vitro* assays have shown the alteration of the development of *Ent. cuniculi*. The disassembly of microtubules caused by albendazole inhibits the formation of mitotic spindles and prevents the nuclear divisions thus producing enlarged and disorganised stages (Colbourn *et al.*, 1994). The deve-

lopment of this species is also inhibited by fumagillin, 5-fluorouracil and sparflloxacin (Beauvais *et al.*, 1994). Patients infected with *Ent. intestinalis* are successfully treated with albendazole as shown by the improvement in symptoms within days of start of therapy and the clearance of spores of the parasite in stools (Sobottka *et al.*, 1995). It is noteworthy that spores of the parasite are still detected in the urine of some treated patients after the improvement of clinical symptoms. The persistence of infected sites could explain the parasitic relapses frequently observed in patients with severe immunodeficiency despite low-dose maintenance therapy with albendazole (Molina *et al.*, 1995). Fumagillin has been used with success in the treatment of keratoconjunctivitis caused by *Ent. bellem* (Diesenhouse *et al.*, 1993). Up to this date no therapy has proven to be effective against *E. bienewisi*.

CONCLUSION

The increasing number of studies on human microsporidiosis have contributed to the better characterization of their agents. They have also improved the diagnosis and the treatment of encephalitozoonoses. However specific treatments of *Enterocytozoon bienewisi* are not yet available. In addition the *in vitro* model of *Ent. bienewisi* is still lacking. No doubt that the obtention of long term culture of this species will facilitate the development of specific immunological tests and facilitate pharmacological studies. The method of isolation of microsporidian spores by flow cytometry developed by Challier *et al.* in 1994 can provide samples of spores of *Ent. bienewisi*. This material is presently selected in our laboratory for the production of antibodies and the infection of cell lines.

The epidemiology of human microsporidiosis is under investigation. Spores of *Ent. bienewisi* have been found in stools of an immunocompetent traveller returning from tropical areas and without HIV infection (Sandfort *et al.*, 1993). Similarly a case of infection by this microsporidia has been reported in a Turkish child living in Germany on her return from a vacation trip in Turkey (Sobottka *et al.*, 1995). These patients presented diarrhoea which resolved without treatment. These observations suggest that *Ent. bienewisi* cause intestinal infections which are spontaneously resolvable in immunocompetent people. The presence of *Ent. bienewisi* in an HIV-negative patient who was treated with cyclosporin after transplant therapy confirms the dramatic involvement of the immunodeficiency in the development and pathogenesis of microsporidiosis (Monneret *et al.*, 1995). It must be pointed out that some cases of microsporidiosis observed in immunocompetent patients are limited to immunoprivileged

sites as shown by corneal stroma infections caused by *Nosema ocularum* (Cali *et al.*, 1991) and *Vittaforma corneae* (Silveira & Canning, 1995). These data confirm the opportunistic character of microsporidian infections.

REFERENCES

- ALDRAS A.M., ORENSTEIN J.M., KOTLER D.P., SHADDUCK J.A. & DIDIER E.S. Detection of microsporidia by indirect immunofluorescence antibody test using polyclonal and monoclonal antibodies. *Journal of Clinical Microbiology*, 1994, 32, 608-612.
- ASMUTH D.M., DE GIROLAMI P.C., FEDERMAN M., EZRATTY C.R., PLESKOW D.K., DESAI G. & WANKE C.A. Clinical features of microsporidiosis in patients with AIDS. *Clinical Infectious Diseases*, 1994, 18, 819-825.
- BEAUVAIS B., SARFATI C., CHALLIER S. & DEROUIN F. *In vitro* model to assess effect of antimicrobial agents on *Encephalitozoon cuniculi*. *Antimicrobial Agents and Chemotherapy*, 1994, 38, 2440-2448.
- CALI A., KOTLER D. & ORENSTEIN J.M. *Septata intestinalis* n.g., n.sp., an intestinal microsporidian associated with chronic diarrhea and dissemination in AIDS patients. *The Journal of Eukaryotic Microbiology*, 1993, 40, 101-112.
- CALI A., MEISLER D.M., LOWDER C.Y., LEMBACH R., AYERS L., TAKVORIAN P., RUTHERFORD I., LONGWORTH D.L., MCMAHON J. & BRYAN R.T. Corneal microsporidiosis: characterization and identification. *The Journal of Protozoology*, 1991, 38, 215-217S.
- CANNING E.U. & LOM J., 1986. The microsporidia of vertebrates, Academic Press, London & New York.
- CHALLIER S., BROWN S., OMBROUCK C., DESPORTES-LIVAGE I., DE NAY D. & GENTILINI M. Flow cytometry as a possible method of isolation of spores of the microsporidian *Enterocytozoon bienewisi*. *The Journal of Eukaryotic Microbiology*, 1994, 41, 27S.
- CHUPP G.L., ALROY J., ADELMAN L.S., BREEN J.C. & SKOLNIK P.R. Myositis due to *Pleistophora* (Microsporidia) in a patient with AIDS. *Clinical Infectious Diseases*, 1992, 16, 15-21.
- COLBOURN N.I., HOLLISTER W.S., CURRY A. & CANNING E.U. Activity of albendazole against *Encephalitozoon cuniculi* *in vitro*. *European Journal of Protistology*, 1994, 30, 211-220.
- DE GIROLAMI P.C., EZRATTY C.R., DESAI G., MC CULLOUGH A., ASMUTH D., WANKE C. & FEDERMAN M. Diagnosis of intestinal microsporidia by examination of stool and duodenal aspirate with Weber's modified trichrome and Uvitex 2B stains. *Journal of Clinical Microbiology*, 1995, 33, 805-810.
- DE GROOTE M.A., VISVESVARA G., WILSON M.L., PIENAZEK N.J., SIEMENDA S.B., DA SILVA A.J., LEITCH G.L., BRYAN R.T. & REEVES R. Polymerase chain reaction and culture confirmation of disseminated *Encephalitozoon cuniculi* in a patient with AIDS: successful therapy with albendazole. *The Journal of Infectious Diseases*, 1995, 171, 1375-1378.
- DEL PRETE G. & ROMAGNINI S. The role of Th1 and Th2 subsets in human infectious diseases. *Trends in Microbiology*, 1994, 2, 4-6.

- DESPORTES I., LE CHARPENTIER Y., GALIAN A., BERNARD F., COCHAND-PRIOLETT B., LAVERGNE A., RAVISSE P. & MODIGLIANI R. Occurrence of a new microsporidian: *Enterocytozoon bienersi* n.g., n.sp. in the enterocytes of a human patient with AIDS. *The Journal of Protozoology*, 1985, 32, 250-254.
- DIDIER E.S. Reactive nitrogen intermediates implicated in the inhibition of *Encephalitozoon cuniculi* (phylum Microspora) replication in murine peritoneal macrophages. *Parasite Immunology*, 1995, 17, 405-412.
- DIDIER E.S., DIDIER P.J., FRIEDBERG D.N., STENSON S.M., ORENSTEIN J.M., YEE R.W., TIO F.O., DAVIS R.M., VOSSBRINCK C., MILLICHAMP N. & SHADDUCK J.A. Isolation and characterization of a new human microsporidian *Encephalitozoon bellem*, n. sp. from three AIDS patients with keratoconjunctivitis. *The Journal of Infectious Diseases*, 1991, 163, 617-621.
- DIDIER E.S., ROHERS L.B., ORENSTEIN J.M., BAKER M.D., VOSSBRINCK C.R., VAN GOOL T., HARTSKEERL R., SOAVE R. & BEAUDET L.M. Characterization of *Encephalitozoon (Septata) intestinalis* isolates cultured from nasal mucosa and bronchoalveolar lavage fluids from two AIDS patients. *Journal of Eukaryotic Microbiology*, 1996, 43, 34-43.
- DIDIER E.S. & SHADDUCK J.A. IFN-gamma and LPS induce murine macrophages to kill *Encephalitozoon cuniculi* in vitro. *Journal of Eukaryotic Microbiology*, 1994, 41, 34S.
- DIDIER E.S., VOSSBRINCK C.R., BAKER M.D., ROGERS L.B., BERTUCCI D.C. & SHADDUCK J.A. Identification and characterization of three *Encephalitozoon cuniculi* strains. *Parasitology*, 1995, 11, 411-421.
- DIDIER E.S., WARNER P.W., DIDIER P.J., ALDRAS A.M., MILLICHAMP N.J., MURPHEY-CORB M., BOHM R. & SHADDUCK J.A. Experimental microsporidiosis in immunocompetent and immunodeficient mice and monkeys. *Folia Parasitologica*, 1994, 41, 1-11.
- DIESENHOUSE M.C., WILSON L.A., CORRENT G.F., VISVESVARA G.S., GROSSNIKLAUS H.E. & BRYAN R.T. Treatment of microsporidial keratoconjunctivitis with topical fumagillin. *American Journal of Ophthalmology*, 1993, 115, 293-298.
- DORE G.J., MARRIOTT D.J., HING M.C., HARKNESS J.L. & FIELD A.S. Disseminated microsporidiosis due to *Septata intestinalis* in nine patients infected with the human immunodeficiency virus: response to therapy with albendazole. *Clinical Infectious Diseases*, 1995, 21, 70-76.
- DOULTREE J.C., MAERZ A.L., RYAN N.J., BAIRD R.W., WRIGHT E., CROWE S.M. & MARSHALL J.A. In vitro growth of the microsporidian *Septata intestinalis* from an AIDS patient with disseminated illness. *Journal of Clinical Microbiology*, 1995, 33, 463-470.
- FEDORKO D., NELSON N.A. & CARTWRIGHT C.P. Identification of microsporidia in stool specimens by using PCR and restriction endonucleases. *Journal of Clinical Microbiology*, 1995, 33, 1739-1741.
- FOISSNER I. & FOISSNER W. *Ciliatosporidium platyophryae* n.g., n.sp. (Microspora *incertae sedis*), a parasite of *Platyophrya terricola* (Ciliophora, Colpodea). *European Journal of Protistology*, 1995, 31, 248-259.
- GUNNARSSON G., HULBURT D., DE GIROLAMI P.C., FEDERMAN M. & WANKE C. Multiorgan microsporidiosis: report of five cases and review. *Clinical Infectious Diseases*, 1995, 21, 37-44.
- HARTSKEERL R.A., VAN GOOL T., SCHUITTEMA A.R., DIDIER E.S. & TERPSTRA W.J. Genetic and immunological characterization of the microsporidian *Septata intestinalis* Cali, Kotler and Orenstein, 1993: reclassification to *Encephalitozoon intestinalis*. *Parasitology*, 1995, 110, 277-285.
- HERMANEK J., KOUDELA B., KUCEROVA Z., DITRICH O. & TRAVNICEK J. Prophylactic and therapeutic immune reconstitution of SCID mice infected with *Encephalitozoon cuniculi*. *Folia Parasitologica*, 1993, 40, 287-291.
- HOLLISTER W.S., CANNING E.U., COLBOURN N.I. & AARONS E.J. *Encephalitozoon cuniculi* isolated from the urine of an AIDS patient, which differs from canine and murine isolates. *The Journal of Eukaryotic Microbiology*, 1995, 42, 367-372.
- HOLLISTER W.S., CANNING E.U., WEIDNER E., FIELD A.S., KENCH J. & MARRIOTT D. J. Development and ultrastructure of *Trachipleistophora hominis* n.g., n.sp., after in vitro isolation from an AIDS patient and inoculation into athymic mice. *Parasitology*, 1995, in press.
- KOKOSKIN E., GYORKOS T.W., CAMUS A., CEDILLOTTE L., PURTILL T. & WARD B. Modified technique for efficient detection of microsporidia. *Journal of Clinical Microbiology*, 1994, 32, 1974-1975.
- KOUDELA B., VITOVEC J., DITRICH O. & TRAVNICEK J. The severe combined immunodeficient mouse for a model for *Encephalitozoon cuniculi* microsporidiosis. *Folia Parasitologica*, 1993, 40, 279-286.
- KOTLER D.P. Gastrointestinal manifestations of human immunodeficiency virus. *Advances in Internal Medicine*, 1995, 40, 197-242.
- LEDFORD D.K., OVERMAN M.D., GONZALO A., CALI A., MESTER W. & LOCKEY R.F. Microsporidiosis myositis in a patient with acquired immunodeficiency syndrome. *Annals of Internal Medicine*, 1985, 102, 628-630.
- MOLINA J.M., OKSENHENDLER E., BEAUVAIS B., SARFATI C., JACCARD A., DEROUIN F. & MODAI J. Disseminated microsporidiosis due to *Septata intestinalis* in patients with AIDS: clinical features and response to albendazole therapy. *The Journal of Infectious Diseases*, 1995, 171, 245-249.
- MONNERET G., RABODONIRINA M., COTTE L., DESPORTES-LIVAGE I., PAULUS S., BASTIEN O., TRONCY J., LACHAUX A., BOIBIEUX A., ROUMANET-DUBOIS F., PIENS M.A. & MOJON M. Détection de spores de microsporidies intestinales dans une population non infectée par le virus de l'immunodéficience humaine. *Annales de Biologie clinique*, 1995, 53, 563-564.
- OMBROUCK C., ROMESTAND B., CORREIA DA COSTA J.M., DESPORTES-LIVAGE I., DATRY A., COSTE F., BOUX G. & GENTILINI M. Use of cross-reactive antigens of the microsporidian *Glugea atherinae* for the possible detection of *Enterocytozoon bienersi* by western blot. *American Journal of Tropical Medicine and Hygiene*, 1995, 52, 89-93.
- OMBROUCK C., DESPORTES-LIVAGE I., ACHBARROU A. & GENTILINI M. Specific detection of the microsporidia *Encephalitozoon intestinalis* in AIDS patients. *Comptes-Rendus de*

- l'Académie des Sciences*, Paris, Sciences de la Vie, 1996, 319, 39-43.
- OMBROUCK C., CICÉRON L., DESPORTES-LIVAGE I. Specific and Rapid Detection of Microsporidia in Stool Specimens from AIDS patients by PCR. *Parasite*, 1996, 3, 85-86.
- RABENECK L., GENTA R.M., GYORKEY F., CLARRIDGE J.E., GYORKEY P. & FOOTE L.W. Observations in the pathological spectrum and clinical course of microsporidiosis in men infected with the human immunodeficiency virus: follow-up study. *Clinical Infectious Diseases*, 1995, 20, 1229-1235.
- SANDFORT J., HANNEMANN A., GELDERBLOM H., STARK K., OWEN R.L. & RUF B. *Enterocytozoon bienewisi* infection in an immunocompetent patient who had acute diarrhea and who was not infected with the human immunodeficiency virus. *Clinical Infectious Diseases*, 1993, 18, 514-516.
- SCHWARTZ D.A., VISVESVARA G.S., DIESENHOUSE M. C., WEBER R., FONT R.L., WILSON L.A., CORENT G., ROSBERGER D.F., GROSSNIKLAUS H., HEWAN-LOWE K. & BRYAN R.T. Ocular pathology of microsporidiosis: role of immunofluorescent antibody for diagnosis of *Encephalitozoon hellem* in biopsies, smears, and intact globes from seven AIDS patients. *American Journal of Ophthalmology*, 1993, 115, 285-292.
- SHUTTEMA A.R.J., HARTSKEERL R.A., VAN GOOL T., LAXMINARAYAN R. & TERPSTRA W.J. Application of the polymerase chain reaction for the diagnosis of microsporidiosis. *AIDS*, 1993, 7 (suppl. 3), S57-61.
- SILVEIRA H. & CANNING E.U. *Vittaforma corneae* N. Comb. for the human microsporidium *Nosema corneum* Shadduck, Meccoli, Davis & Font, 1990, based on its ultrastructure in the liver of experimentally infected athymic mice. *The Journal of Eukaryotic Microbiology*, 1995, 42, 158-165.
- SOBOTTKA I., ALBRECHT H., SCHÄFER H., SCHOTTELUS J., VISVESVARA G.S., LAUFS R. & SCHWARTZ D.A. Disseminated *Encephalitozoon (Septata) intestinalis* infection in a patient with AIDS: novel diagnostic approaches and autopsy-confirmed parasitological cure following treatment with albendazole. *Journal of Clinical Microbiology*, 1995, 33, 2948-2952.
- SOBOTTKA I., ALBRECHT H., SCHOTTELUS J., SCHMETZ C., BENTFIELD M., LAUFS R. & SCHWARTZ D.A. Self-limited traveller's diarrhoea due to a dual infection with *Enterocytozoon bienewisi* and *Cryptosporidium parvum* in an immunocompetent HIV-negative Child. *European Journal of Clinical Microbiology and Infectious Diseases*, 1995, 14, 919-920.
- SPRAGUE V. *Nosema connori* n. sp., a microsporidian parasite of man. *Transactions of the American Microscopical Society*, 1974, 93, 400-403.
- VAN GOOL T., CANNING E.U. & GILIS H. *Septata intestinalis* frequently isolated from stool of AIDS patients with a new cultivation method. *Parasitology*, 1994, 109, 281-289.
- VAN GOOL T., SNIJDERS F., EEF TINCK-SCHATTENKERK J.K.M., VAN DER BERG WEERMAN M.A., BARTELSMAN J.F.W., BRUINS J.J.M., CANNING E.U. & DANKERT J.A. Diagnosis of microsporidian infections in HIV-infected individuals with a new rapid fluorescence technique. *Journal of Clinical Pathology*, 1993, 46, 694-699.
- VISVESVARA G.S., LEITCH G. J., DA SILVA A.J., CROPPA G.P., MOURA H., WALLACE S., SLEMENDA S.B., SCHWARTZ D.A., MOSS D., BRYAN R.T. & PIENIAZK N.J. Polyclonal and monoclonal antibody and PCR-amplified small-subunit rRNA identification of a microsporidian, *Encephalitozoon hellem*, isolated from an AIDS patient with disseminated infection. *Journal of Clinical Microbiology*, 1995, 32, 2760-2768.
- VISVESVARA G.S., DA SILVA A.J., CROPPA G.P., PIENIAZEK N.J., LEITCH G.J., FERGUSSON D., DE MOURA H., WALLACE S., SLEMENDA S.B., TYRRELL I., MOORE D.F. & MEADOR J. *In vitro* culture and serologic and molecular identification of *Septata intestinalis* isolated from urine of a patient with AIDS. *Journal of Clinical Microbiology*, 1995, 33, 930-936.
- VOSSBRINCK C.R., MADDUX J.V., FRIEDMAN S., DEBRUNNER-VOSSBRINCK B.A. & WOESE C.R. Ribosomal RNA sequence suggests microsporidia are extremely ancient eukaryotes. *Nature*, 1987, 326, 411-414.
- VOULDOUKIS I., MAZIER D., DEBRÉ P. & MOSSALAYI M.D. Nitric oxide and human infectious disease. *Research in Immunology*, in press.
- WEBER R., BRYAN R.T., OWEN R.L., WILCOX C.M., GORELKIN L. & VISVESVARA G.S. Improved light-microscopical detection of microsporidia spores in stool and duodenal aspirates. *New England Journal of Medicine and Hygiene*, 1992, 326, 161-166.
- WEBER R., BRYAN R.T., SCHWARTZ D.A. & OWEN R.L. Human microsporidian infections. *Clinical and Microbiological Review*, 1994, 7, 426-461.
- WEISS L.M., ZHU X., KEOHANE E., CALI A., TANOWITZ H.B. & WITTNER M. Polymerase chain reaction identification of microsporidia using ribosomal RNA. *AIDS*, 1993, 7 (suppl. 3), S62-63.
- ZHU X., WITTNER M., TANOWITZ H.B., CALI A. & WEISS L.M. Ribosomal RNA sequences of *Enterocytozoon bienewisi*, *Septata intestinalis* and *Ameson michaelis*: phylogenetic construction and structural correspondence. *The Journal of Eukaryotic Microbiology*, 1994, 41, 204-209.
- ZIERDT C.H., GILL V.J. & ZIERDT W.S. Detection of microsporidian spores in clinical samples by indirect fluorescent-antibody assay using whole-cell antisera to *Encephalitozoon cuniculi* and *Encephalitozoon hellem*. *Journal of Clinical Microbiology*, 1993, 31, 3071-3074.