References

1. Skalski JH, Kottom TJ, Limper AH. Pathobiology of *Pneumocystis* pneumonia: life cycle, cell wall and cell signal transduction. *FEMS Yeast Res*. 2015;15.
2. Brown GD, Denning DW, Gow NA, et al. Hidden killers: human fungal infections. *Sci Transl Med*. 2012;4:165rv113.
3. Vargas SL, Ponce CA, Luchsinger V, et al. Detection of *Pneumocystis carinii* f. sp. hominis and viruses in presumably immunocompetent infants who died in the hospital or in the community. *J Infect Dis*. 2005;191:122–126.
4. Eddens T, Campfield BT, Serody K, et al. A novel CD4(+) T cell-dependent murine model of *Pneumocystis*-driven asthma-like pathology. *Am J Respir Crit Care Med*. 2016;194:807–820.
5. Wickramasekaran RN, Jewell MP, Sorvillo F, et al. The changing trends and profile of pneumocystosis mortality in the United States, 1999-2014. *Mycoses*. 2017;60:607–615.
6. Morris A, Norris KA. Colonization by *Pneumocystis jirovecii* and its role in disease. *Clin Microbiol Rev*. 2012;25:297–317.
7. Ma L, Chen Z, Huang da W, et al. Genome analysis of three *Pneumocystis* species reveals adaptation mechanisms to life exclusively in mammalian hosts. *Nat Commun*. 2016;7:10740.
8. Cisse OH, Pagni M, Hauser PM. De novo assembly of the *Pneumocystis jirovecii* genome from a single bronchoalveolar lavage fluid specimen from a patient. *MBio*. 2012;4:e428–e512.
9. Sesterhenn TM, Slaven BE, Keely SP, et al. Sequence and structure of the linear mitochondrial genome of *Pneumocystis carinii*. *Mol Genet Genomics*. 2010;283:63–72.
10. Ma L, Huang DW, Cuomo CA, et al. Sequencing and characterization of the complete mitochondrial genomes of three *Pneumocystis* species provide new insights into divergence between human and rodent *Pneumocystis*. *FASEB J*. 2013;27:1962–1972.
11. Redhead SA, Cushion MT, Frenkel JK, et al. *Pneumocystis* and *Trypanosoma cruzi:* nomenclature and typifications. *J Eukaryot Microbiol*. 2006;53:2–11.
12. Stringer JR, Beard CB, Miller RF, et al. A new name *(Pneumocystis jiroveci)* for *Pneumocystis* from humans. *Emerg Infect Dis*. 2002;8:891–896.
13. Schildgen V, Mai S, Khalfaoui S, et al. *Pneumocystis jirovecii* can be productively cultured in differentiated CuFi-8 airway cells. *MBio*. 2014;5:e01186-01114.
14. Cushion MT, Collins MS, Linke MJ. Biofilm formation by *Pneumocystis* spp. *Eukaryot Cell*. 2009;8:197–206.
15. Burgess JW, Kottom TJ, Limper AH. *Pneumocystis carinii* exhibits a conserved meiotic control pathway. *Infect Immun*. 2008;76:417–425.
16. Kutty G, Achaz G, Maldarelli F, et al. Characterization of the meiosis-specific recombinase Dmc1 of *Pneumocystis*. *J Infect Dis*. 2010;202:1920–1929.
17. Almeida JM, Cisse OH, Fonseca A, et al. Comparative genomics suggests primary homothallism of *Pneumocystis* species. *MBio*. 2015;6.
18. Cushion MT, Linke MJ, Ashbaugh A, et al. Echinocandin treatment of *Pneumocystis* pneumonia in rodent models depletes cysts leaving trophic burdens that cannot transmit the infection. *PLoS ONE*. 2010;5:e8524.
19. Schmatz DM, Powles M, McFadden DC, et al. Treatment and prevention of *Pneumocystis carinii* pneumonia and further elucidation of the *P. carinii* life cycle with 1,3-beta-glucan synthesis inhibitor L-671,329. *J Protozool*. 1991;38:151S–153S.
20. Kaneshiro ES. Sterol metabolism in the opportunistic pathogen *Pneumocystis:* advances and new insights. *Lipids*. 2004;39:753–761.
21. Hauser PM, Burdet FX, Cisse OH, et al. Comparative genomics suggests that the fungal pathogen *Pneumocystis* is an obligate parasite scavenging amino acids from its host's lungs. *PLoS ONE*. 2010;5:e15152.
22. Cushion MT, Smulian AG, Slaven BE, et al. Transcriptome of *Pneumocystis carinii* during fulminate infection: carbohydrate metabolism and the concept of a compatible parasite. *PLoS ONE*. 2007;2:e423.
23. De Stefano JA, Myers JD, Du Pont D, et al. Cell wall antigens of *Pneumocystis carinii* trophozoites and cysts: purification and carbohydrate analysis of these glycoproteins. *J Eukaryot Microbiol*. 1998;45:334–343.
24. Villegas LR, Kottom TJ, Limper AH. Chitinases in *Pneumocystis carinii* pneumonia. *Med Microbiol Immunol*. 2012;201:337–348.
25. Porollo A, Meller J, Joshi Y, et al. Analysis of current antifungal agents and their targets within the *Pneumocystis carinii* genome. *Curr Drug Targets*. 2012;13:1575–1585.
26. Cushion MT, Collins MS, Sesterhenn T, et al. Functional characterization of *Pneumocystis carinii* inositol transporter 1. *MBio*. 2016;7.
27. Stringer JR. Antigenic variation in *Pneumocystis*. *J Eukaryot Microbiol*. 2007;54:8–13.
28. Linke MJ, Sunkin SM, Andrews RP, et al. Expression, structure, and location of epitopes of the major surface glycoprotein of *Pneumocystis carinii* f. sp. *carinii*. *Clin Diagn Lab Immunol*. 1998;5:50–57.
29. Gigliotti F, Hughes WT. Passive immunoprophylaxis with specific monoclonal antibody confers partial protection against *Pneumocystis carinii* pneumonitis in animal models. *J Clin Invest*. 1988;81:1666–1668.
30. Theus SA, Walzer PD. Adoptive transfer of specific lymphocyte populations sensitized to the major surface glycoprotein of *Pneumocystis carinii* decreases organism burden while increasing survival rate in the rat. *J Eukaryot Microbiol*. 1997;44:23S–24S.
31. Theus SA, Smulian AG, Steele P, et al. Immunization with the major surface glycoprotein of *Pneumocystis carinii* elicits a protective response. *Vaccine*. 1998;16:1149–1157.
32. Pottratz ST, Paulsrud J, Smith JS, et al. *Pneumocystis carinii* attachment to cultured lung cells by *Pneumocystis* gp 120, a fibronectin binding protein. *J Clin Invest*. 1991;88:403–407.
33. Ezekowitz RA, Williams DJ, Koziel H, et al. Uptake of *Pneumocystis carinii* mediated by the macrophage mannose receptor. *Nature*. 1991;351:155–158.
34. Kottom TJ, Kennedy CC, Limper AH. Pneumocystis PCINT1, a molecule with integrin-like features that mediates organism adhesion to fibronectin. *Mol Microbiol*. 2008;67:747–761.
35. Limper AH, Standing JE, Hoffman OA, et al. Vitronectin binds to *Pneumocystis carinii* and mediates organism attachment to cultured lung epithelial cells. *Infect Immun*. 1993;61:4302–4309.
36. McCormack FX, Festa AL, Andrews RP, et al. The carbohydrate recognition domain of surfactant protein A mediates binding to the major surface glycoprotein of *Pneumocystis carinii*. *Biochemistry*. 1997;36:8092–8099.
37. O'Riordan DM, Standing JE, Kwon KY, et al. Surfactant protein D interacts with *Pneumocystis carinii* and mediates organism adherence to alveolar macrophages. *J Clin Invest*. 1995;95:2699–2710.
38. Bishop LR, Helman D, Kovacs JA. Discordant antibody and cellular responses to *Pneumocystis* major surface glycoprotein variants in mice. *BMC Immunol*. 2012;13:39.
39. Kutty G, Ma L, Kovacs JA. Characterization of the expression site of the major surface glycoprotein of human-derived *Pneumocystis carinii*. *Mol Microbiol*. 2001;42:183–193.
40. Schmid-Siegert E, Richard S, Luraschi A, et al. Mechanisms of surface antigenic variation in the human pathogenic fungus *Pneumocystis jirovecii*. *MBio*. 2017;8.
41. Daly KR, Koch JV, Shire NJ, et al. Human immunodeficiency virus-infected patients with prior *Pneumocystis* pneumonia exhibit increased serologic reactivity to several major surface glycoprotein clones. *Clin Vaccine Immunol*. 2006;13:1071–1078.
42. Walzer PD, Djawe K, Levin L, et al. Long-term serologic responses to the *Pneumocystis jirovecii* major surface glycoprotein in HIV-positive individuals with and without *P. jirovecii* infection. *J Infect Dis*. 2009;199:1335–1344.
43. Daly K, Koch J, Respaldiza N, et al. Geographical variation in serological responses to recombinant *Pneumocystis jirovecii* major surface glycoprotein antigens. *Clin Microbiol Infect*. 2009;15:937–942.
44. Tipirneni R, Daly KR, Jarlsberg LG, et al. Healthcare worker occupation and immune response to *Pneumocystis jirovecii*. *Emerg Infect Dis*. 2009;15:1590–1597.
45. Schaffzin JK, Sunkin SM, Stringer JR. A new family of *Pneumocystis carinii* genes related to those encoding the major surface glycoprotein. *Curr Genet*. 1999;35:134–143.
46. Kutty G, Kovacs JA. A single-copy gene encodes Kex1, a serine endoprotease of *Pneumocystis jiroveci*. *Infect Immun*. 2003;71:571–574.
47. Gigliotti F, Haidaris CG, Wright TW, et al. Passive intranasal monoclonal antibody prophylaxis against murine *Pneumocystis carinii* pneumonia. *Infect Immun*. 2002;70:1069–1074.
48. Kling HM, Shipley TW, Patil SP, et al. Relationship of *Pneumocystis jiroveci* humoral immunity to prevention of colonization and chronic obstructive pulmonary disease in a primate model of HIV infection. *Infect Immun*. 2010;78:4320–4330.
49. Feng Y, Guo S, Jiang T, et al. Active immunization against *Pneumocystis carinii* with p55-v3 DNA vaccine in rats. *Can J Microbiol*. 2011;57:375–381.
50. Ma L, Kutty G, Jia Q, et al. Characterization of variants of the gene encoding the p55 antigen in *Pneumocystis* from rats and mice. *J Med Microbiol*. 2003;52:955–960.
51. Walzer PD. Immunological features of *Pneumocystis carinii* infection in humans. *Clin Diagn Lab Immunol*. 1999;6:149–155.
52. Montes-Cano MA, Chabe M, Fontillon-Alberdi M, et al. Vertical transmission of *Pneumocystis jirovecii* in humans. *Emerg Infect Dis*. 2009;15:125–127.
53. Vargas SL, Hughes WT, Santolaya ME, et al. Search for primary infection by *Pneumocystis carinii* in a cohort of normal, healthy infants. *Clin Infect Dis*. 2001;32:855–861.
54. Larsen HH, von Linstow ML, Lundgren B, et al. Primary *Pneumocystis* infection in infants hospitalized with acute respiratory tract infection. *Emerg Infect Dis*. 2007;13:66–72.
55. Peglow SL, Smulian AG, Linke MJ, et al. Serologic responses to *Pneumocystis carinii* antigens in health and disease. *J Infect Dis*. 1990;161:296–306.
56. Hernandez D, Rodriguez N, Martinez C, et al. *Leishmania braziliensis* causing visceral leishmaniasis in a patient with human immunodeficiency virus infection, identified with the aid of the polymerase chain reaction. *Trans R Soc Trop Med Hyg*. 1993;87:627–628.
57. Linke MJ, Ashbaugh AD, Demland JA, et al. *Pneumocystis murina* colonization in immunocompetent surfactant protein A deficient mice following environmental exposure. *Respir Res*. 2009;10:10.
58. Ahn IE, Jerussi T, Farooqui M, et al. Atypical *Pneumocystis jirovecii* pneumonia in previously untreated patients with CLL on single-agent ibrutinib. *Blood*. 2016;128:1940–1943.
59. Demanche C, Wanert F, Barthelemy M, et al. Molecular and serological evidence of *Pneumocystis* circulation in a social organization of healthy macaques *(Macaca fascicularis)*. *Microbiology*. 2005;151:3117–3125.
60. Davis JL, Welsh DA, Beard CB, et al. *Pneumocystis* colonisation is common among hospitalised HIV infected patients with non-*Pneumocystis* pneumonia. *Thorax*. 2008;63:329–334.
61. Shipley TW, Kling HM, Morris A, et al. Persistent *Pneumocystis* colonization leads to the development of chronic obstructive pulmonary disease in a non human primate model of AIDS. *J Infect Dis*. 2010;202:302–312.
62. Masur H, Michelis MA, Greene JB, et al. An outbreak of community-acquired *Pneumocystis carinii* pneumonia: initial manifestation of cellular immune dysfunction. *N Engl J Med*. 1981;305:1431–1438.
63. Miller RF, Huang L, Walzer PD. *Pneumocystis* pneumonia associated with human immunodeficiency virus. *Clin Chest Med*. 2013;34:229–241.
64. Buchacz K, Lau B, Jing Y, et al. Incidence of AIDS-defining opportunistic infections in a multicohort analysis of HIV-infected persons in the United States and Canada, 2000-2010. *J Infect Dis*. 2016;214:862–872.
65. Lowe DM, Rangaka MX, Gordon F, et al. *Pneumocystis jirovecii* pneumonia in tropical and low and middle income countries: a systematic review and meta-regression. *PLoS ONE*. 2013;8:e69969.
66. Lanaspa M, O'Callaghan-Gordo C, Machevo S, et al. High prevalence of *Pneumocystis jirovecii* pneumonia among Mozambican children <5 years of age admitted to hospital with clinical severe pneumonia. *Clin Microbiol Infect*. 2015;21:1018.e1009–1018.e1015.
67. Matsumura Y, Shindo Y, Iinuma Y, et al. Clinical characteristics of *Pneumocystis* pneumonia in non-HIV patients and prognostic factors including microbiological genotypes. *BMC Infect Dis*. 2011;11:76.
68. de Boer MG, Kroon FP, le Cessie S, et al. Risk factors for *Pneumocystis jirovecii* pneumonia in kidney transplant recipients and appraisal of strategies for selective use of chemoprophylaxis. *Transpl Infect Dis*. 2011;13:559–569.
69. Maertens J, Cesaro S, Maschmeyer G, et al. ECIL guidelines for preventing *Pneumocystis jirovecii* pneumonia in patients with haematological malignancies and stem cell transplant recipients. *J Antimicrob Chemother*. 2016;71:2397–2404.
70. Wolfe RM, Peacock JE Jr. *Pneumocystis* pneumonia and the rheumatologist: which patients are at risk and how can PCP be prevented? *Curr Rheumatol Rep*. 2017;19:35.
71. Siegel JD, Rhinehart E, Jackson M, et al. 2007 Guideline for isolation precautions: preventing transmission of infectious agents in health care settings. *Am J Infect Control*. 2007;35:S65–S164.
72. Walzer P. The ecology of *Pneumocystis:* perspectives, recollections, and future research opportunities. *J Eukaryot Microbiol*. 2013;60:634–645.
73. Navin TR, Rimland D, Lennox JL, et al. Risk factors for community-acquired pneumonia among persons infected with human immunodeficiency virus. *J Infect Dis*. 2000;181:158–164.
74. Dohn MN, White ML, Vigdorth EM, et al. Geographic clustering of *Pneumocystis carinii* pneumonia in patients with HIV infection. *Am J Respir Crit Care Med*. 2000;162:1617–1621.

75. Morris AM, Swanson M, Ha H, et al. Geographic distribution of human immunodeficiency virus-associated *Pneumocystis carinii* pneumonia in San Francisco. *Am J Respir Crit Care Med*. 2000;162:1622–1626.
76. Miller RF, Evans HE, Copas AJ, et al. Climate and genotypes of *Pneumocystis jirovecii*. *Clin Microbiol Infect*. 2007;13:445–448.
77. Djawe K, Levin L, Swartzman A, et al. Environmental risk factors for *Pneumocystis* pneumonia hospitalizations in HIV patients. *Clin Infect Dis*. 2013;56:74–81.
78. Walzer PD, Kim CK, Linke MJ, et al. Outbreaks of *Pneumocystis carinii* pneumonia in colonies of immunodeficient mice. *Infect Immun*. 1989;57:62–70.
79. Icenhour CR, Rebholz SL, Collins MS, et al. Widespread occurrence of *Pneumocystis carinii* in commercial rat colonies detected using targeted PCR and oral swabs. *J Clin Microbiol*. 2001;39:3437–3441.
80. de Boer MG, de Fijter JW, Kroon FP. Outbreaks and clustering of *Pneumocystis* pneumonia in kidney transplant recipients: a systematic review. *Med Mycol*. 2011;49:673–680.
81. Choukri F, Menotti J, Sarfati C, et al. Quantification and spread of *Pneumocystis jirovecii* in the surrounding air of patients with *Pneumocystis* pneumonia. *Clin Infect Dis*. 2010;51:259–265.
82. Djawe K, Daly KR, Vargas SL, et al. Seroepidemiological study of *Pneumocystis jirovecii* infection in healthy infants in Chile using recombinant fragments of the *P. jirovecii* major surface glycoprotein. *Int J Infect Dis*. 2010;14:e1060–e1066.
83. Djawe K, Huang L, Daly KR, et al. Serum antibody levels to the *Pneumocystis jirovecii* major surface glycoprotein in the diagnosis of *P. jirovecii* pneumonia in HIV+ patients. *PLoS ONE*. 2010;5:e14259.
84. Gingo MR, Lucht L, Daly KR, et al. Serologic responses to *Pneumocystis* proteins in HIV patients with and without *Pneumocystis jirovecii* pneumonia. *J Acquir Immune Defic Syndr*. 2011;57:190–196.
85. Blount RJ, Jarlsberg LG, Daly KR, et al. Serologic responses to recombinant *Pneumocystis jirovecii* major surface glycoprotein among Ugandan patients with respiratory symptoms. *PLoS ONE*. 2012;7:e51545.
86. Walzer PD, Kim CK, Cushion MT. *Pneumocystis carinii*. In: Walzer PD, Genta RM, eds. *Parasitic Infection in the Compromised Host*. New York: Marcel Dekker; 1989:83–178.
87. Pottratz ST, Weir AL. Attachment of *Pneumocystis carinii* to primary cultures of rat alveolar epithelial cells. *Exp Cell Res*. 1995;221:357–362.
88. Kottom TJ, Kohler JR, Thomas CF Jr, et al. Lung epithelial cells and extracellular matrix components induce expression of *Pneumocystis carinii STE20,* a gene complementing the mating and pseudohyphal growth defects of STE20 mutant yeast. *Infect Immun*. 2003;71:6463–6471.
89. Porollo A, Sesterhenn TM, Collins MS, et al. Comparative genomics of *Pneumocystis* species suggests the absence of genes for myo-inositol synthesis and reliance on inositol transport and metabolism. *MBio*. 2014;5:e01834.
90. Koziel H, Kim S, Reardon C, et al. Enhanced in vivo human immunodeficiency virus-1 replication in the lungs of human immunodeficiency virus-infected persons with *Pneumocystis carinii* pneumonia. *Am J Respir Crit Care Med*. 1999;160:2048–2055.
91. Limper AH, Edens M, Anders RA, et al. *Pneumocystis carinii* inhibits cyclin-dependent kinase activity in lung epithelial cells. *J Clin Invest*. 1998;101:1148–1155.
92. Yu ML, Limper AH. *Pneumocystis carinii* induces ICAM-1 expression in lung epithelial cells through a TNF-alpha-mediated mechanism. *Am J Physiol*. 1997;273:L1103–L1111.
93. Hoving JC, Kolls JK. New advances in understanding the host immune response to *Pneumocystis*. *Curr Opin Microbiol*. 2017;40:65–71.
94. Linke MJ, Harris CE, Korfhagen TR, et al. Immunosuppressed surfactant protein A-deficient mice have increased susceptibility to *Pneumocystis carinii* infection. *J Infect Dis*. 2001;183:943–952.
95. Atochina EN, Gow AJ, Beck JM, et al. Delayed clearance of *Pneumocystis carinii* infection, increased inflammation, and altered nitric oxide metabolism in lungs of surfactant protein-D knockout mice. *J Infect Dis*. 2004;189:1528–1539.
96. Rapaka RR, Ricks DM, Alcorn JF, et al. Conserved natural IgM antibodies mediate innate and adaptive immunity against the opportunistic fungus *Pneumocystis murina*. *J Exp Med*. 2010;207:2907–2919.
97. Martin-Garrido I, Carmona EM, Specks U, et al. *Pneumocystis* pneumonia in patients treated with rituximab. *Chest*. 2013;144:258–265.
98. Chen W, Gigliotti F, Harmsen AG. Latency is not an inevitable outcome of infection with *Pneumocystis carinii*. *Infect Immun*. 1993;61:5406–5409.
99. Harmsen AG, Chen W, Gigliotti F. Active immunity to *Pneumocystis carinii* reinfection in T-cell-depleted mice. *Infect Immun*. 1995;63:2391–2395.
100. de la Rua NM, Samuelson DR, Charles TP, et al. CD4(+) T-cell-independent secondary immune responses to *Pneumocystis* pneumonia. *Front Immunol*. 2016;7:178.
101. Kling HM, Shipley TW, Patil SP, et al. Relationship of *Pneumocystis jiroveci* humoral immunity to prevention of colonization and chronic obstructive pulmonary disease in a primate model of HIV infection. *Infect Immun*. 2010;78:4320–4330.
102. Harmsen AG, Chen W, Gigliotti F. Active immunity to *Pneumocystis carinii* reinfection in T-cell-depleted mice. *Infect Immun*. 1995;63:2391–2395.
103. Garvy BA, Wiley JA, Gigliotti F, et al. Protection against *Pneumocystis carinii* pneumonia by antibodies generated from either T helper 1 or T helper 2 responses. *Infect Immun*. 1997;65:5052–5056.
104. Zheng M, Ramsay AJ, Robichaux MB, et al. CD4+ T cell-independent DNA vaccination against opportunistic infections. *J Clin Invest*. 2005;115:3536–3544.
105. Zheng M, Rapaka RR, Yu AC, et al. Role of interleukin-23-dependent antifungal immune responses in dendritic cell-vaccinated mice. *Infect Immun*. 2011;79:3778–3783.
106. Tesini BL, Wright TW, Malone JE, et al. Immunization with *Pneumocystis* cross-reactive antigen 1 (Pca1) protects mice against *Pneumocystis* pneumonia and generates antibody to *Pneumocystis jirovecii*. *Infect Immun*. 2017;85.
107. Lund FE, Hollifield M, Schuer K, et al. B cells are required for generation of protective effector and memory CD4 cells in response to *Pneumocystis* lung infection. *J Immunol*. 2006;176:6147–6154.
108. Moir S, Fauci AS. Pathogenic mechanisms of B-lymphocyte dysfunction in HIV disease. *J Allergy Clin Immunol*. 2008;122:12–19, quiz 20-1.
109. Djawe K, Daly KR, Levin L, et al. Humoral immune responses to *Pneumocystis jirovecii* antigens in HIV-infected and uninfected young children with *Pneumocystis* pneumonia. *PLoS ONE*. 2013;8:e82783.
110. Laursen AL, Jensen BN, Andersen PL. Local antibodies against *Pneumocystis carinii* in bronchoalveolar lavage fluid. *Eur Respir J*. 1994;7:679–685.
111. Jalil A, Moja P, Lambert C, et al. Decreased production of local immunoglobulin A to *Pneumocystis carinii* in bronchoalveolar lavage fluid from human immunodeficiency virus-positive patients. *Infect Immun*. 2000;68:1054–1060.
112. Blount RJ, Daly KR, Fong S, et al. Effects of clinical and environmental factors on bronchoalveolar antibody responses to *Pneumocystis jirovecii*: a prospective cohort study of HIV+ patients. *PLoS ONE*. 2017;12:e0180212.
113. Furuta T, Fujita M, Machii K, et al. Fatal spontaneous pneumocystosis in nude rats. *Lab Anim Sci*. 1993;43:551–556.
114. Shellito J, Suzara VV, Blumenfeld W, et al. A new model of *Pneumocystis carinii* infection in mice selectively depleted of helper T lymphocytes. *J Clin Invest*. 1990;85:1686–1693.
115. Theus SA, Andrews RP, Steele P, et al. Adoptive transfer of lymphocytes sensitized to the major surface glycoprotein of *Pneumocystis carinii* confers protection in the rat. *J Clin Invest*. 1995;95:2587–2593.
116. Harmsen AG, Stankiewicz M. Requirement for CD4+ cells in resistance to *Pneumocystis carinii* pneumonia in mice. *J Exp Med*. 1990;172:937–945.
117. Steele C, Zheng M, Young E, et al. Increased host resistance against *Pneumocystis carinii* pneumonia in gammadelta T-cell-deficient mice: protective role of gamma interferon and CD8(+) T cells. *Infect Immun*. 2002;70:5208–5215.
118. Kolls JK, Habetz S, Shean MK, et al. IFN-gamma and CD8+ T cells restore host defenses against *Pneumocystis carinii* in mice depleted of CD4+ T cells. *J Immunol*. 1999;162:2890–2894.
119. Wiley JA, Harmsen AG. CD40 ligand is required for resolution of *Pneumocystis carinii* pneumonia in mice. *J Immunol*. 1995;155:3525–3529.
120. Beck JM, Blackmon MB, Rose CM, et al. T cell costimulatory molecule function determines susceptibility to infection with *Pneumocystis carinii* in mice. *J Immunol*. 2003;171:1969–1977.
121. McAllister F, Steele C, Zheng M, et al. In vitro effector activity of *Pneumocystis murina*-specific T-cytotoxic-1 CD8+ T cells: role of granulocyte-macrophage colony-stimulating factor. *Infect Immun*. 2005;73:7450–7457.
122. Levy J, Espanol-Boren T, Thomas C, et al. Clinical spectrum of X-linked hyper-IgM syndrome. *J Pediatr*. 1997;131:47–54.
123. Garvy BA, Qureshi MH. Delayed inflammatory response to *Pneumocystis carinii* infection in neonatal mice is due to an inadequate lung environment. *J Immunol*. 2000;165:6480–6486.
124. Qureshi MH, Garvy BA. Neonatal T cells in an adult lung environment are competent to resolve *Pneumocystis carinii* pneumonia. *J Immunol*. 2001;166:5704–5711.
125. Phair J, Munoz A, Detels R, et al. The risk of *Pneumocystis carinii* pneumonia among men infected with human immunodeficiency virus type 1. Multicenter AIDS Cohort Study Group. *N Engl J Med*. 1990;322:161–165.
126. Kulke MH, Vance EA. *Pneumocystis carinii* pneumonia in patients receiving chemotherapy for breast cancer. *Clin Infect Dis*. 1997;25:215–218.
127. Smith DK, Neal JJ, Holmberg SD. Unexplained opportunistic infections and CD4+ T-lymphocytopenia without HIV infection. An investigation of cases in the United States. The Centers for Disease Control Idiopathic CD4+ T-lymphocytopenia Task Force. *N Engl J Med*. 1993;328:373–379.
128. Mansharamani NG, Balachandran D, Vernovsky I, et al. Peripheral blood CD4+ T-lymphocyte counts during *Pneumocystis carinii* pneumonia in immunocompromised patients without HIV infection. *Chest*. 2000;118:712–720.
129. Agostini C, Adami F, Poulter LW, et al. Role of bronchoalveolar lavage in predicting survival of patients with human immunodeficiency virus infection. *Am J Respir Crit Care Med*. 1997;156:1501–1507.
130. Iriart X, Witkowski B, Cassaing S, et al. Alveolar and blood T lymphocyte profiles in *Pneumocystis jirovecii*-positive patients: effects of HIV status. *J Infect Dis*. 2011;204:544–553.
131. White PL, Backx M, Barnes RA. Diagnosis and management of *Pneumocystis jirovecii* infection. *Expert Rev Anti Infect Ther*. 2017;15:435–447.
132. Carmona EM, Limper AH. Update on the diagnosis and treatment of *Pneumocystis* pneumonia. *Ther Adv Respir Dis*. 2011;5:41–59.
133. Reid AB, Chen SC, Worth LJ. *Pneumocystis jirovecii* pneumonia in non-HIV-infected patients: new risks and diagnostic tools. *Curr Opin Infect Dis*. 2011;24:534–544.
134. Hugle B, Solomon M, Harvey E, et al. *Pneumocystis jiroveci* pneumonia following rituximab treatment in Wegener's granulomatosis. *Arthritis Care Res (Hoboken)*. 2010;62:1661–1664.
135. Sepkowitz KA. Opportunistic infections in patients with and patients without acquired immunodeficiency syndrome. *Clin Infect Dis*. 2002;34:1098–1107.
136. Yale SH, Limper AH. *Pneumocystis carinii* pneumonia in patients without acquired immunodeficiency syndrome: associated illness and prior corticosteroid therapy. *Mayo Clin Proc*. 1996;71:5–13.
137. Godeau B, Coutant-Perronne V, Le Thi Huong D, et al. *Pneumocystis carinii* pneumonia in the course of connective tissue disease: report of 34 cases. *J Rheumatol*. 1994;21:246–251.
138. Graham BS, Tucker WS Jr. Opportunistic infections in endogenous Cushing's syndrome. *Ann Intern Med*. 1984;101:334–338.
139. Abernathy-Carver KJ, Fan LL, Boguniewicz M, et al. *Legionella* and *Pneumocystis* pneumonias in asthmatic children on high doses of systemic steroids. *Pediatr Pulmonol*. 1994;18:135–138.
140. Limper AH, Hoyte JS, Standing JE. The role of alveolar macrophages in *Pneumocystis carinii* degradation and clearance from the lung. *J Clin Invest*. 1997;99:2110–2117.
141. Hanano R, Kaufmann SH. Activation of pulmonary macrophages is not sufficient for in vivo containment of *Pneumocystis carinii*. *J Eukaryot Microbiol*. 1997;44:17S.
142. Steele C, Marrero L, Swain S, et al. Alveolar macrophage-mediated killing of *Pneumocystis carinii* f. sp. *muris* involves molecular recognition by the dectin-1 beta-glucan receptor. *J Exp Med*. 2003;198:1677–1688.
143. Zhang C, Wang SH, Lasbury ME, et al. Toll-like receptor 2 mediates alveolar macrophage response to *Pneumocystis murina*. *Infect Immun*. 2006;74:1857–1864.
144. Ding K, Shibui A, Wang Y, et al. Impaired recognition by Toll-like receptor 4 is responsible for exacerbated murine *Pneumocystis* pneumonia. *Microbes Infect*. 2005;7:195–203.
145. Vassallo R, Kottom TJ, Standing JE, et al. Vitronectin and fibronectin function as glucan binding proteins augmenting macrophage responses to *Pneumocystis carinii*. *Am J Respir Cell Mol Biol*. 2001;25:203–211.
146. Vassallo R, Standing JE, Limper AH. Isolated *Pneumocystis carinii* cell wall glucan provokes lower respiratory tract inflammatory responses. *J Immunol*. 2000;164:3755–3763.
147. Laursen AL, Moller B, Rungby J, et al. *Pneumocystis carinii*-induced activation of the respiratory burst in human monocytes and macrophages. *Clin Exp Immunol*. 1994;98:196–202.
148. Lasbury ME, Merali S, Durant PJ, et al. Polyamine-mediated apoptosis of alveolar macrophages during *Pneumocystis* pneumonia. *J Biol Chem*. 2007;282:11009–11020.

149. Evans HM, Simpson A, Shen S, et al. The trophic life cycle stage of the opportunistic fungal pathogen *Pneumocystis murina* hinders the ability of dendritic cells to stimulate CD4(+) T cell responses. *Infect Immun*. 2017;85.
150. Deckman JM, Kurkjian CJ, McGillis JP, et al. *Pneumocystis* infection alters the activation state of pulmonary macrophages. *Immunobiology*. 2017;222:188–197.
151. Nandakumar V, Hebrink D, Jenson P, et al. Differential macrophage polarization from *Pneumocystis* in immunocompetent and immunosuppressed hosts: potential adjunctive therapy during pneumonia. *Infect Immun*. 2017;85.
152. Kurkjian C, Hollifield M, Lines JL, et al. Alveolar macrophages in neonatal mice are inherently unresponsive to *Pneumocystis murina* infection. *Infect Immun*. 2012;80:2835–2846.
153. Zhang C, Wang SH, Liao CP, et al. Downregulation of PU.1 leads to decreased expression of Dectin-1 in alveolar macrophages during *Pneumocystis* pneumonia. *Infect Immun*. 2010;78:1058–1065.
154. Inoue M, Moriwaki Y, Arikawa T, et al. Cutting edge: critical role of intracellular osteopontin in antifungal innate immune responses. *J Immunol*. 2011;186:19–23.
155. Kandil O, Fishman JA, Koziel H, et al. Human immunodeficiency virus type 1 infection of human macrophages modulates the cytokine response to *Pneumocystis carinii*. *Infect Immun*. 1994;62:644–650.
156. Koziel H, Eichbaum Q, Kruskal BA, et al. Reduced binding and phagocytosis of *Pneumocystis carinii* by alveolar macrophages from persons infected with HIV-1 correlates with mannose receptor downregulation. *J Clin Invest*. 1998;102:1332–1344.
157. Fraser IP, Takahashi K, Koziel H, et al. *Pneumocystis carinii* enhances soluble mannose receptor production by macrophages. *Microbes Infect*. 2000;2:1305–1310.
158. Stehle SE, Rogers RA, Harmsen AG, et al. A soluble mannose receptor immunoadhesin enhances phagocytosis of *Pneumocystis carinii* by human polymorphonuclear leukocytes in vitro. *Scand J Immunol*. 2000;52:131–137.
159. Carmona EM, Lamont JD, Xue A, et al. *Pneumocystis* cell wall beta-glucan stimulates calcium-dependent signaling of IL-8 secretion by human airway epithelial cells. *Respir Res*. 2010;11:95.
160. Evans SE, Kottom TJ, Pagano RE, et al. Primary alveolar epithelial cell surface membrane microdomain function is required for *Pneumocystis* beta-glucan-induced inflammatory responses. *Innate Immun*. 2012;18:709–716.
161. Carmona EM, Kottom TJ, Hebrink DM, et al. Glycosphingolipids mediate *Pneumocystis* cell wall beta-glucan activation of the IL-23/IL-17 axis in human dendritic cells. *Am J Respir Cell Mol Biol*. 2012;47:50–59.
162. Limper AH. Tumor necrosis factor alpha-mediated host defense against *Pneumocystis carinii*. *Am J Respir Cell Mol Biol*. 1997;16:110–111.
163. Chen W, Havell EA, Moldawer LL, et al. Interleukin 1: an important mediator of host resistance against *Pneumocystis carinii*. *J Exp Med*. 1992;176:713–718.
164. Chen W, Havell EA, Gigliotti F, et al. Interleukin-6 production in a murine model of *Pneumocystis carinii* pneumonia: relation to resistance and inflammatory response. *Infect Immun*. 1993;61:97–102.
165. Beck JM, Liggitt HD, Brunette EN, et al. Reduction in intensity of *Pneumocystis carinii* pneumonia in mice by aerosol administration of gamma interferon. *Infect Immun*. 1991;59:3859–3862.
166. Paine R 3rd, Preston AM, Wilcoxen S, et al. Granulocyte-macrophage colony-stimulating factor in the innate immune response to *Pneumocystis carinii* pneumonia in mice. *J Immunol*. 2000;164:2602–2609.
167. Ruan S, McKinley L, Zheng M, et al. Interleukin-12 and host defense against murine *Pneumocystis* pneumonia. *Infect Immun*. 2008;76:2130–2137.
168. Rudner XL, Happel KI, Young EA, et al. Interleukin-23 (IL-23)-IL-17 cytokine axis in murine *Pneumocystis carinii* infection. *Infect Immun*. 2007;75:3055–3061.
169. Qureshi MH, Harmsen AG, Garvy BA. IL-10 modulates host responses and lung damage induced by *Pneumocystis carinii* infection. *J Immunol*. 2003;170:1002–1009.
170. Ieki R, Furuta T, Asano S, et al. Effect of recombinant human granulocyte colony-stimulating factor on *Pneumocystis carinii* infection in nude mice. *Jpn J Exp Med*. 1989;59:51–58.
171. Wright TW, Johnston CJ, Harmsen AG, et al. Analysis of cytokine mRNA profiles in the lungs of *Pneumocystis carinii*-infected mice. *Am J Respir Cell Mol Biol*. 1997;17:491–500.
172. Perenboom RM, Beckers P, Van Der Meer JW, et al. Pro-inflammatory cytokines in lung and blood during steroid-induced *Pneumocystis carinii* pneumonia in rats. *J Leukoc Biol*. 1996;60:710–715.
173. Perenboom RM, van Schijndel AC, Beckers P, et al. Cytokine profiles in bronchoalveolar lavage fluid and blood in HIV-seronegative patients with *Pneumocystis carinii* pneumonia. *Eur J Clin Invest*. 1996;26:159–166.
174. Perenboom RM, Sauerwein RW, Beckers P, et al. Cytokine profiles in bronchoalveolar lavage fluid and blood in HIV-seropositive patients with *Pneumocystis carinii* pneumonia. *Eur J Clin Invest*. 1997;27:333–339.
175. Travis WD, Pittaluga S, Lipschik GY, et al. Atypical pathologic manifestations of *Pneumocystis carinii* pneumonia in the acquired immune deficiency syndrome. Review of 123 lung biopsies from 76 patients with emphasis on cysts, vascular invasion, vasculitis, and granulomas. *Am J Surg Pathol*. 1990;14:615–625.
176. D'Angelo E, Calderini E, Robatto FM, et al. Lung and chest wall mechanics in patients with acquired immunodeficiency syndrome and severe *Pneumocystis carinii* pneumonia. *Eur Respir J*. 1997;10:2343–2350.
177. Stansell JD, Osmond DH, Charlebois E, et al. Predictors of *Pneumocystis carinii* pneumonia in HIV-infected persons. Pulmonary Complications of HIV Infection Study Group. *Am J Respir Crit Care Med*. 1997;155:60–66.
178. Wang Z, Foye A, Chang Y, et al. Inhibition of surfactant activity by *Pneumocystis carinii* organisms and components in vitro. *Am J Physiol Lung Cell Mol Physiol*. 2005;288:L1124–L1131.
179. Su TH, Natarajan V, Kachel DL, et al. Functional impairment of bronchoalveolar lavage phospholipids in early *Pneumocystis carinii* pneumonia in rats. *J Lab Clin Med*. 1996;127:263–271.
180. Hoffman AG, Lawrence MG, Ognibene FP, et al. Reduction of pulmonary surfactant in patients with human immunodeficiency virus infection and *Pneumocystis carinii* pneumonia. *Chest*. 1992;102:1730–1736.
181. Lipschik GY, Treml JF, Moore SD, et al. *Pneumocystis carinii* glycoprotein A inhibits surfactant phospholipid secretion by rat alveolar type II cells. *J Infect Dis*. 1998;177:182–187.
182. Sternberg RI, Whitsett JA, Hull WM, et al. *Pneumocystis carinii* alters surfactant protein A concentrations in bronchoalveolar lavage fluid. *J Lab Clin Med*. 1995;125:462–469.
183. Beers MF, Atochina EN, Preston AM, et al. Inhibition of lung surfactant protein B expression during *Pneumocystis carinii* pneumonia in mice. *J Lab Clin Med*. 1999;133:423–433.
184. Atochina EN, Beck JM, Scanlon ST, et al. *Pneumocystis carinii* pneumonia alters expression and distribution of lung collectins SP-A and SP-D. *J Lab Clin Med*. 2001;137:429–439.
185. Roths JB, Sidman CL. Both immunity and hyperresponsiveness to *Pneumocystis carinii* result from transfer of CD4+ but not CD8+ T cells into severe combined immunodeficiency mice. *J Clin Invest*. 1992;90:673–678.
186. Wright TW, Gigliotti F, Finkelstein JN, et al. Immune-mediated inflammation directly impairs pulmonary function, contributing to the pathogenesis of *Pneumocystis carinii* pneumonia. *J Clin Invest*. 1999;104:1307–1317.
187. Wright TW, Johnston CJ, Harmsen AG, et al. Chemokine gene expression during *Pneumocystis carinii*-driven pulmonary inflammation. *Infect Immun*. 1999;67:3452–3460.
188. Wright TW, Notter RH, Wang Z, et al. Pulmonary inflammation disrupts surfactant function during *Pneumocystis carinii* pneumonia. *Infect Immun*. 2001;69:758–764.
189. Bhagwat SP, Gigliotti F, Xu H, et al. Contribution of T cell subsets to the pathophysiology of *Pneumocystis*-related immunorestitution disease. *Am J Physiol Lung Cell Mol Physiol*. 2006;291:L1256–L1266.
190. Hori S, Carvalho TL, Demengeot J. CD25+CD4+ regulatory T cells suppress CD4+ T cell-mediated pulmonary hyperinflammation driven by *Pneumocystis carinii* in immunodeficient mice. *Eur J Immunol*. 2002;32:1282–1291.
191. Gigliotti F, Crow EL, Bhagwat SP, et al. Sensitized CD8+ T cells fail to control organism burden but accelerate the onset of lung injury during *Pneumocystis carinii* pneumonia. *Infect Immun*. 2006;74:6310–6316.
192. Meissner NN, Lund FE, Han S, et al. CD8 T cell-mediated lung damage in response to the extracellular pathogen *Pneumocystis* is dependent on MHC class I expression by radiation-resistant lung cells. *J Immunol*. 2005;175:8271–8279.
193. McKinley L, Logar AJ, McAllister F, et al. Regulatory T cells dampen pulmonary inflammation and lung injury in an animal model of *Pneumocystis* pneumonia. *J Immunol*. 2006;177:6215–6226.
194. Swain SD, Meissner NN, Harmsen AG. CD8 T cells modulate CD4 T-cell and eosinophil-mediated pulmonary pathology in *Pneumocystis* pneumonia in B-cell-deficient mice. *Am J Pathol*. 2006;168:466–475.
195. Swain SD, Wright TW, Degel PM, et al. Neither neutrophils nor reactive oxygen species contribute to tissue damage during *Pneumocystis* pneumonia in mice. *Infect Immun*. 2004;72:5722–5732.
196. Thullen TD, Ashbaugh AD, Daly KR, et al. Sensitized splenocytes result in deleterious cytokine cascade and hyperinflammatory response in rats with *Pneumocystis* pneumonia despite the presence of corticosteroids. *Infect Immun*. 2004;72:757–765.
197. Mason GR, Hashimoto CH, Dickman PS, et al. Prognostic implications of bronchoalveolar lavage neutrophilia in patients with *Pneumocystis carinii* pneumonia and AIDS. *Am Rev Respir Dis*. 1989;139:1336–1342.
198. Benfield TL, Vestbo J, Junge J, et al. Prognostic value of interleukin-8 in AIDS-associated *Pneumocystis carinii* pneumonia. *Am J Respir Crit Care Med*. 1995;151:1058–1062.
199. Benfield TL, Kharazmi A, Larsen CG, et al. Neutrophil chemotactic activity in bronchoalveolar lavage fluid of patients with AIDS-associated *Pneumocystis carinii* pneumonia. *Scand J Infect Dis*. 1997;29:367–371.
200. Benfield TL, Lundgren B, Levine SJ, et al. The major surface glycoprotein of *Pneumocystis carinii* induces release and gene expression of interleukin-8 and tumor necrosis factor alpha in monocytes. *Infect Immun*. 1997;65:4790–4794.
201. Tachado SD, Zhang J, Zhu J, et al. *Pneumocystis*-mediated IL-8 release by macrophages requires coexpression of mannose receptors and TLR2. *J Leukoc Biol*. 2007;81:205–211.
202. The National Institutes of Health-University of California Expert Panel for Corticosteroids as Adjunctive Therapy for Pneumocystis Pneumonia. Consensus statement on the use of corticosteroids as adjunctive therapy for *Pneumocystis* pneumonia in the acquired immunodeficiency syndrome. *N Engl J Med*. 1990;323:1500–1504.
203. Benfield TL, van Steenwijk R, Nielsen TL, et al. Interleukin-8 and eicosanoid production in the lung during moderate to severe *Pneumocystis carinii* pneumonia in AIDS: a role of interleukin-8 in the pathogenesis of *P. carinii* pneumonia. *Respir Med*. 1995;89:285–290.
204. Huang ZB, Eden E. Effect of corticosteroids on IL1 beta and TNF alpha release by alveolar macrophages from patients with AIDS and *Pneumocystis carinii* pneumonia. *Chest*. 1993;104:751–755.
205. Benfield TL, Schattenkerk JK, Hofmann B, et al. Differential effect on serum neopterin and serum beta 2-microglobulin is induced by treatment in *Pneumocystis carinii* pneumonia. *J Infect Dis*. 1994;169:1170–1173.
206. Dichter JR, Lundgren JD, Nielsen TL, et al. *Pneumocystis carinii* pneumonia in HIV-infected patients: effect of steroid therapy on surfactant level. *Respir Med*. 1999;93:373–378.
207. Huang L. Clinical presentation and diagnosis of *Pneumocystis* pneumonia in HIV-infected patients. In: Walzer PD, Cushion MT, eds. *Pneumocystis Pneumonia*. New York: Marcell Dekker; 2005:349–406.
208. Haverkos HW. Assessment of therapy for *Pneumocystis carinii* pneumonia. PCP Therapy Project Group. *Am J Med*. 1984;76:501–508.
209. Kovacs JA, Hiemenz JW, Macher AM, et al. *Pneumocystis carinii* pneumonia: a comparison between patients with the acquired immunodeficiency syndrome and patients with other immunodeficiencies. *Ann Intern Med*. 1984;100:663–671.
210. Chen HX, Ryan PA, Ferguson RP, et al. Characteristics of acquired immunodeficiency syndrome in older adults. *J Am Geriatr Soc*. 1998;46:153–156.
211. Laing R, Brettle R, Leen C, et al. Features and outcome of *Pneumocystis carinii* pneumonia according to risk category for HIV infection. *Scand J Infect Dis*. 1997;29:57–61.
212. Ng VL, Yajko DM, Hadley WK. Extrapulmonary pneumocystosis. *Clin Microbiol Rev*. 1997;10:401–418.
213. Murdoch DM, Venter WD, Van Rie A, et al. Immune reconstitution inflammatory syndrome (IRIS): review of common infectious manifestations and treatment options. *AIDS Res Ther*. 2007;4:9.
214. Ratnam I, Chiu C, Kandala NB, et al. Incidence and risk factors for immune reconstitution inflammatory syndrome in an ethnically diverse HIV type 1-infected cohort. *Clin Infect Dis*. 2006;42:418–427.
215. Hirsch HH, Kaufmann G, Sendi P, et al. Immune reconstitution in HIV-infected patients. *Clin Infect Dis*. 2004;38:1159–1166.
216. Takahashi T, Nakamura T, Iwamoto A. Reconstitution of immune responses to *Pneumocystis carinii* pneumonia in

patients with HIV infection who receive highly active antiretroviral therapy. *Res Commun Mol Pathol Pharmacol.* 2002;112:59–67.

217. Benfield TL, Prento P, Junge J, et al. Alveolar damage in AIDS-related *Pneumocystis carinii* pneumonia. *Chest.* 1997;111:1193–1199.
218. Sato M, Ito S, Ogura M, et al. Atypical *Pneumocystis jiroveci* pneumonia with multiple nodular granulomas after rituximab for refractory nephrotic syndrome. *Pediatr Nephrol.* 2013;28:145–149.
219. Fahy JV, Chin DP, Schnapp LM, et al. Effect of aerosolized pentamidine prophylaxis on the clinical severity and diagnosis of *Pneumocystis carinii* pneumonia. *Am Rev Respir Dis.* 1992;146:844–848.
220. Tumeh SS, Belville JS, Pugatch R, et al. Ga-67 scintigraphy and computed tomography in the diagnosis of *Pneumocystis carinii* pneumonia in patients with AIDS. A prospective comparison. *Clin Nucl Med.* 1992;17:387–394.
221. Goldenberg DM, Sharkey RM, Udem S, et al. Immunoscintigraphy of *Pneumocystis carinii* pneumonia in AIDS patients. *J Nucl Med.* 1994;35:1028–1034.
222. Benson CA, Spear J, Hines D, et al. Combined APACHE II score and serum lactate dehydrogenase as predictors of in-hospital mortality caused by first episode *Pneumocystis carinii* pneumonia in patients with acquired immunodeficiency syndrome. *Am Rev Respir Dis.* 1991;144:319–323.
223. White PL, Posso RB, Gorton RL, et al. An evaluation of the performance of the Dynamiker(R) Fungus (1-3)-β-D-Glucan Assay to assist in the diagnosis of *Pneumocystis* pneumonia. *Med Mycol.* 2018;56:778–781.
224. Rose SR, Vallabhajosyula S, Velez MG, et al. The utility of bronchoalveolar lavage beta-D-glucan testing for the diagnosis of invasive fungal infections. *J Infect.* 2014;69:278–283.
225. Kroe DM, Kirsch CM, Jensen WA. Diagnostic strategies for *Pneumocystis carinii* pneumonia. *Semin Respir Infect.* 1997;12:70–78.
226. Cadranel J, Gillet-Juvin K, Antoine M, et al. Site-directed bronchoalveolar lavage and transbronchial biopsy in HIV-infected patients with pneumonia. *Am J Respir Crit Care Med.* 1995;152:1103–1106.
227. Huang L, Hecht FM, Stansell JD, et al. Suspected *Pneumocystis carinii* pneumonia with a negative induced sputum examination. Is early bronchoscopy useful? *Am J Respir Crit Care Med.* 1995;151:1866–1871.
228. Gruden JF, Huang L, Turner J, et al. High-resolution CT in the evaluation of clinically suspected *Pneumocystis carinii* pneumonia in AIDS patients with normal, equivocal, or nonspecific radiographic findings. *AJR Am J Roentgenol.* 1997;169:967–975.
229. Huang L, Stansell J, Osmond D, et al. Performance of an algorithm to detect *Pneumocystis carinii* pneumonia in symptomatic HIV-infected persons. Pulmonary Complications of HIV Infection Study Group. *Chest.* 1999;115:1025–1032.
230. Montaner J, Zala C. The role of the laboratory in the diagnosis and management of AIDS-related *Pneumocystis carinii* pneumonia. In: Sattler F, Walzer PD, eds. *Pneumocystis carinii*. London: Bailliere Tindall; 1995:471–485.
231. Aslanzadeh J, Stelmach PS. Detection of *Pneumocystis carinii* with direct fluorescence antibody and calcofluor white stain. *Infection.* 1996;24:248–250.
232. Smulian AG, Linke MJ, Cushion MT, et al. Analysis of *Pneumocystis carinii* organism burden, viability and antigens in bronchoalveolar lavage fluid in AIDS patients with pneumocystosis: correlation with disease severity. *AIDS.* 1994;8:1555–1562.
233. Huggett JF, Taylor MS, Kocjan G, et al. Development and evaluation of a real-time PCR assay for detection of *Pneumocystis* jirovecii DNA in bronchoalveolar lavage fluid of HIV-infected patients. *Thorax.* 2008;63:154–159.
234. Robberts FJ, Liebowitz LD, Chalkley LJ. Polymerase chain reaction detection of *Pneumocystis* jiroveci: evaluation of 9 assays. *Diagn Microbiol Infect Dis.* 2007;58:385–392.
235. Alvarez-Martinez MJ, Miro JM, Valls ME, et al. Sensitivity and specificity of nested and real-time PCR for the detection of *Pneumocystis jiroveci* in clinical specimens. *Diagn Microbiol Infect Dis.* 2006;56:153–160.
236. Skelly MJ, Holzman RS, Merali S. S-adenosylmethionine levels in the diagnosis of *Pneumocystis carinii* pneumonia in patients with HIV infection. *Clin Infect Dis.* 2008;46:467–471.
237. de Boer MG, Gelinck LB, van Zelst BD, et al. β-D-glucan and S-adenosylmethionine serum levels for the diagnosis of *Pneumocystis* pneumonia in HIV-negative patients: a prospective study. *J Infect.* 2011;62:93–100.
238. Birriel JA Jr, Adams JA, Saldana MA, et al. Role of flexible bronchoscopy and bronchoalveolar lavage in the diagnosis of pediatric acquired immunodeficiency syndrome-related pulmonary disease. *Pediatrics.* 1991;87:897–899.
239. Helweg-Larsen J. *Pneumocystis jiroveci.* Applied molecular microbiology, epidemiology and diagnosis. *Dan Med Bull.* 2004;51:251–273.
240. Masur H, Shelhamer J. Empiric outpatient management of HIV-related pneumonia: economical or unwise? *Ann Intern Med.* 1996;124:451–453.
241. Miller RF, Millar AB, Weller IV, et al. Empirical treatment without bronchoscopy for *Pneumocystis carinii* pneumonia in the acquired immunodeficiency syndrome. *Thorax.* 1989;44:559–564.
242. Glassroth J. Empiric diagnosis of *Pneumocystis carinii* pneumonia. Questions of accuracy and equity. *Am J Respir Crit Care Med.* 1995;152:1433–1434.
243. Parada JP, Deloria-Knoll M, Chmiel JS, et al. Relationship between health insurance and medical care for patients hospitalized with human immunodeficiency virus-related *Pneumocystis carinii* pneumonia, 1995-1997: medicaid, bronchoscopy, and survival. *Clin Infect Dis.* 2003;37:1549–1555.
244. Gracia JD, Miravitlles M, Mayordomo C, et al. Empiric treatments impair the diagnostic yield of BAL in HIV-positive patients. *Chest.* 1997;111:1180–1186.
245. Lee N, Lawrence D, Patel B, et al. HIV-related *Pneumocystis jirovecii* pneumonia managed with caspofungin and veno-venous extracorporeal membrane oxygenation rescue therapy. *BMJ Case Rep.* 2017;2017.
246. Walzer PD, Evans HE, Copas AJ, et al. Early predictors of mortality from *Pneumocystis jirovecii* pneumonia in HIV-infected patients: 1985-2006. *Clin Infect Dis.* 2008;46:625–633.
247. Fei MW, Kim EJ, Sant CA, et al. Predicting mortality from HIV-associated *Pneumocystis* pneumonia at illness presentation: an observational cohort study. *Thorax.* 2009;64:1070–1076.
248. Monnet X, Vidal-Petiot E, Osman D, et al. Critical care management and outcome of severe *Pneumocystis* pneumonia in patients with and without HIV infection. *Crit Care.* 2008;12:R28.
249. Li MC, Lee NY, Lee CC, et al. *Pneumocystis jiroveci* pneumonia in immunocompromised patients: delayed diagnosis and poor outcomes in non-HIV-infected individuals. *J Microbiol Immunol Infect.* 2012.
250. Keely SP, Stringer JR, Baughman RP, et al. Genetic variation among *Pneumocystis carinii hominis* isolates in recurrent pneumocystosis. *J Infect Dis.* 1995;172:595–598.
251. Dohn MN, Baughman RP, Vigdorth EM, et al. Equal survival rates for first, second, and third episodes of *Pneumocystis carinii* pneumonia in patients with acquired immunodeficiency syndrome. *Arch Intern Med.* 1992;152:2465–2470.
252. Metersky ML, Colt HG, Olson LK, et al. AIDS-related spontaneous pneumothorax. Risk factors and treatment. *Chest.* 1995;108:946–951.
253. Stansell JD, Hopewell PC. *Pneumocystis carinii* pneumonia: risk factors, clinical presentation and natural history. In: Sattler FR, Walzer PD, eds. *Pneumocystis carinii*. London: Bailliere Tindall; 1995:449–459.
254. Panel on Opportunistic Infections in HIV-Infected Adults and Adolescents. Guidelines for Prevention and Treatment of Opportunistic Infections in HIV-Infected Adults and Adolescents: Recommendations from the Centers for Disease Control and Prevention, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. http://aidsinfo.nih.gov/contentfiles/lvguidelines/adult_oi.pdf. Accessed December 17, 2017.
255. Maschmeyer G, Helweg-Larsen J, Pagano L, et al. ECIL guidelines for treatment of *Pneumocystis jirovecii* pneumonia in non-HIV-infected haematology patients. *J Antimicrob Chemother.* 2016;71:2405–2413.
256. Hughes WT, Feldman S, Chaudhary SC, et al. Comparison of pentamidine isethionate and trimethoprim-sulfamethoxazole in the treatment of *Pneumocystis carinii* pneumonia. *J Pediatr.* 1978;92:285–291.
257. Wharton JM, Coleman DL, Wofsy CB, et al. Trimethoprim-sulfamethoxazole or pentamidine for *Pneumocystis carinii* pneumonia in the acquired immunodeficiency syndrome. A prospective randomized trial. *Ann Intern Med.* 1986;105:37–44.
258. Sattler FR, Cowan R, Nielsen DM, et al. Trimethoprim-sulfamethoxazole compared with pentamidine for treatment of *Pneumocystis carinii* pneumonia in the acquired immunodeficiency syndrome. A prospective, noncrossover study. *Ann Intern Med.* 1988;109:280–287.
259. Klein NC, Duncanson FP, Lenox TH, et al. Trimethoprim-sulfamethoxazole versus pentamidine for *Pneumocystis* carinii pneumonia in AIDS patients: results of a large prospective randomized treatment trial. *AIDS.* 1992;6:301–305.
260. Larsen HH, Masur H, Kovacs JA. Current regimens for treatment and prophylaxis of *Pneumocystis jirovecii* pneumonia. In: Walzer PD, Cushion MT, eds. *Pneumocystis Pneumonia*. New York: Marcel Dekker; 2005:505–538.
261. Stein DS, Stevens RC. Treatment-associated toxicities: incidence and mechanisms. In: Sattler FR, Walzer PD, eds. *Pneumocystis carinii*. London: Baillliere Tindall; 1995:505–530.
262. Carr A, Swanson C, Penny R, et al. Clinical and laboratory markers of hypersensitivity to trimethoprim-sulfamethoxazole in patients with *Pneumocystis carinii* pneumonia and AIDS. *J Infect Dis.* 1993;167:180–185.
263. Veenstra J, Veugelers PJ, Keet IP, et al. Rapid disease progression in human immunodeficiency virus type 1-infected individuals with adverse reactions to trimethoprim-sulfamethoxazole prophylaxis. *Clin Infect Dis.* 1997;24:936–941.
264. Velazquez H, Perazella MA, Wright FS, et al. Renal mechanism of trimethoprim-induced hyperkalemia. *Ann Intern Med.* 1993;119:296–301.
265. Joos B, Blaser J, Opravil M, et al. Monitoring of co-trimoxazole concentrations in serum during treatment of *Pneumocystis carinii* pneumonia. *Antimicrob Agents Chemother.* 1995;39:2661–2666.
266. Caumes E, Roudier C, Rogeaux O, et al. Effect of corticosteroids on the incidence of adverse cutaneous reactions to trimethoprim-sulfamethoxazole during treatment of AIDS-associated *Pneumocystis carinii* pneumonia. *Clin Infect Dis.* 1994;18:319–323.
267. Leoung GS, Stanford JF, Giordano MF, et al. Trimethoprim-sulfamethoxazole (TMP-SMZ) dose escalation versus direct rechallenge for *Pneumocystis carinii* pneumonia prophylaxis in human immunodeficiency virus-infected patients with previous adverse reaction to TMP-SMZ. *J Infect Dis.* 2001;184:992–997.
268. Safrin S, Finkelstein DM, Feinberg J, et al. Comparison of three regimens for treatment of mild to moderate *Pneumocystis carinii* pneumonia in patients with AIDS. A double-blind, randomized, trial of oral trimethoprim-sulfamethoxazole, dapsone-trimethoprim, and clindamycin-primaquine. ACTG 108 Study Group. *Ann Intern Med.* 1996;124:792–802.
269. Lee BL, Medina I, Benowitz NL, et al. Dapsone, trimethoprim, and sulfamethoxazole plasma levels during treatment of *Pneumocystis* pneumonia in patients with the acquired immunodeficiency syndrome (AIDS). Evidence of drug interactions. *Ann Intern Med.* 1989;110:606–611.
270. Toma E, Fournier S, Dumont M, et al. Clindamycin/primaquine versus trimethoprim-sulfamethoxazole as primary therapy for *Pneumocystis carinii* pneumonia in AIDS: a randomized, double-blind pilot trial. *Clin Infect Dis.* 1993;17:178–184.
271. Hughes W, Leoung G, Kramer F, et al. Comparison of atovaquone (566C80) with trimethoprim-sulfamethoxazole to treat *Pneumocystis carinii* pneumonia in patients with AIDS. *N Engl J Med.* 1993;328:1521–1527.
272. Dohn MN, Weinberg WG, Torres RA, et al. Oral atovaquone compared with intravenous pentamidine for *Pneumocystis carinii* pneumonia in patients with AIDS. Atovaquone Study Group. *Ann Intern Med.* 1994;121:174–180.
273. Rosenberg DM, McCarthy W, Slavinsky J, et al. Atovaquone suspension for treatment of *Pneumocystis carinii* pneumonia in HIV-infected patients. *AIDS.* 2001;15:211–214.
274. Benfield T, Atzori C, Miller RF, et al. Second-line salvage treatment of AIDS-associated *Pneumocystis jirovecii* pneumonia: a case series and systematic review. *J Acquir Immune Defic Syndr.* 2008;48:63–67.
275. Smego RA Jr, Nagar S, Maloba B, et al. A meta-analysis of salvage therapy for *Pneumocystis carinii* pneumonia. *Arch Intern Med.* 2001;161:1529–1533.
276. Black JR, Feinberg J, Murphy RL, et al. Clindamycin and primaquine therapy for mild-to-moderate episodes of *Pneumocystis carinii* pneumonia in patients with AIDS: AIDS Clinical Trials Group 044. *Clin Infect Dis.* 1994;18:905–913.
277. Noskin GA, Murphy RL, Black JR, et al. Salvage therapy with clindamycin/primaquine for *Pneumocystis carinii* pneumonia. *Clin Infect Dis.* 1992;14:183–188.
278. Comtois R, Pouliot J, Vinet B, et al. Higher pentamidine levels in AIDS patients with hypoglycemia and azotemia during treatment of *Pneumocystis carinii* pneumonia. *Am Rev Respir Dis.* 1992;146:740–744.
279. Sattler FR, Jelliffe RW. Pharmacokinetic and pharmacodynamic considerations for drug dosing in the treatment of *Pneumocystis carinii* pneumonia. In: Walzer PD, ed. *Pneumocystis carinii Pneumonia*. New York: Marcel Dekker; 1994:467–486.

280. Lidman C, Bronner U, Gustafsson LL, et al. Plasma pentamidine concentrations vary between individuals with *Pneumocystis carinii* pneumonia and the drug is actively secreted by the kidney. *J Antimicrob Chemother*. 1994;33:803–810.
281. Kleyman TR, Roberts C, Ling BN. A mechanism for pentamidine-induced hyperkalemia: inhibition of distal nephron sodium transport. *Ann Intern Med*. 1995;122:103–106.
282. Briel M, Bucher HC, Boscacci R, et al. Adjunctive corticosteroids for *Pneumocystis jiroveci* pneumonia in patients with HIV-infection. *Cochrane Database Syst Rev*. 2006;(3):CD006150.
283. Bozzette SA, Morton SC. Reconsidering the use of adjunctive corticosteroids in *Pneumocystis* pneumonia? *J Acquir Immune Defic Syndr Hum Retrovirol*. 1995;8:345–347.
284. Walmsley S, Levinton C, Brunton J, et al. A multicenter randomized double-blind placebo-controlled trial of adjunctive corticosteroids in the treatment of *Pneumocystis carinii* pneumonia complicating the acquired immune deficiency syndrome. *J Acquir Immune Defic Syndr Hum Retrovirol*. 1995;8:348–357.
285. Injean P, Eells SJ, Wu H, et al. A systematic review and meta-analysis of the data behind current recommendations for corticosteroids in non-HIV-related PCP: knowing when you are on shaky foundations. *Transplant Direct*. 2017;3:e137.
286. Wang LI, Liang H, Ye LI, et al. Adjunctive corticosteroids for the treatment of *Pneumocystis jiroveci* pneumonia in patients with HIV: a meta-analysis. *Exp Ther Med*. 2016;11:683–687.
287. Kazanjian P, Armstrong W, Hossler PA, et al. *Pneumocystis carinii* mutations are associated with duration of sulfa or sulfone prophylaxis exposure in AIDS patients. *J Infect Dis*. 2000;182:551–557.
288. Stein CR, Poole C, Kazanjian P, et al. Sulfa use, dihydropteroate synthase mutations, and *Pneumocystis jirovecii* pneumonia. *Emerg Infect Dis*. 2004;10:1760–1765.
289. Crothers K, Beard CB, Turner J, et al. Severity and outcome of HIV-associated *Pneumocystis* pneumonia containing *Pneumocystis jirovecii* dihydropteroate synthase gene mutations. *AIDS*. 2005;19:801–805.
290. Valerio A, Tronconi E, Mazza F, et al. Genotyping of *Pneumocystis jiroveci* pneumonia in Italian AIDS patients. Clinical outcome is influenced by dihydropteroate synthase and not by internal transcribed spacer genotype. *J Acquir Immune Defic Syndr*. 2007;45:521–528.
291. Alvarez-Martinez MJ, Moreno A, Miro JM, et al. *Pneumocystis jirovecii* pneumonia in Spanish HIV-infected patients in the combined antiretroviral therapy era: prevalence of dihydropteroate synthase mutations and prognostic factors of mortality. *Diagn Microbiol Infect Dis*. 2008;62:34–43.
292. Kazanjian P, Armstrong W, Hossler PA, et al. *Pneumocystis carinii* cytochrome b mutations are associated with atovaquone exposure in patients with AIDS. *J Infect Dis*. 2001;183:819–822.
293. Wang J, Wright TW, Gigliotti F. Immune modulation as adjunctive therapy for *Pneumocystis* pneumonia. *Interdiscip Perspect Infect Dis*. 2011;2011:918038.
294. Bhagwat SP, Wright TW, Gigliotti F. Anti-CD3 antibody decreases inflammation and improves outcome in a murine model of *Pneumocystis* pneumonia. *J Immunol*. 2010;184:497–502.
295. Kuramochi T, Hioki K, Ito M. *Pneumocystis carinii* cysts are susceptible to inactivation by chemical disinfectants. *Exp Anim*. 1997;46:241–245.
296. Kaneshiro ES, Maiorano JN. Survival and infectivity of *Pneumocystis carinii* outside the mammalian host. *J Eukaryot Microbiol*. 1996;43:35S.

270 Microsporidiosis

Louis M. Weiss

SHORT VIEW SUMMARY

Definition
- Microsporidia are obligate eukaryotic intracellular pathogens related to fungi.
- Taxonomy of the phylum is based on ultrastructural descriptions of the spores and life cycle.
- Molecular phylogeny, based on ribosomal RNA sequences, is also used for classification.

Epidemiology
- The phylum Microsporidia contains at least 1500 species distributed into more than 200 genera.
- Several different genera and species of microsporidia cause disease in humans, including *Nosema, Vittaforma, Pleistophora, Encephalitozoon, Enterocytozoon, Trachipleistophora, Anncaliia, Tubulinosema, Endoreticulatus,* and *Microsporidium.*
- Microsporidiosis occurs in both immunocompromised and immune-competent hosts.
- These pathogens can be transmitted by food or water and are likely zoonotic.
- Diarrhea or keratoconjunctivitis are the most common presenting manifestations of infection, but infection can occur in any organ system.
- An increasing incidence is reported in transplant recipients. Although multiple sites may be infected, the most common manifestation of reported disease leading to diagnosis is infection of either native or transplanted kidneys. Therapy with albendazole or fumagillin has been successful.
- Infection in solid-organ transplant recipients may be derived from the organ donor.

Diagnosis
- Diagnosis can be made by finding characteristic spores in body fluids (e.g., stool, urine, conjunctival scraping, etc., using stains such as chromotrope 2R or Uvitex 2B).
- Definitive identification of the microsporidia causing an infection can be done using ultrastructural examination or molecular techniques.
- Patients with diarrhea or keratoconjunctivitis should have urine examined to look for disseminated infection.
- Species-specific diagnosis is useful for guiding treatment.

Therapy
- Systemic albendazole and fumagillin are active therapeutic agents for the treatment of microsporidiosis.
- Topical fumagillin is useful for microsporidian keratoconjunctivitis.
- Immune restoration, such as combination antiretroviral treatment in patients with acquired immunodeficiency syndrome, often results in resolution of infection.

Prevention
- There are limited data on effective preventive strategies for microsporidiosis, but the most effective prophylaxis is restoration of immune function in immunocompromised hosts.
- The usual sanitary measures that prevent contamination of food and water will decrease the chance of infection.
- Hand washing and general hygienic habits reduce the chance of contamination of the conjunctiva and cornea with microsporidian spores.

The Microsporidia are a group of obligate eukaryotic intracellular parasites that were recognized more than 150 years ago when a parasite of silkworms, *Nosema bombycis,* which causes the disease pébrine in these economically important insects, was described.[1–5] Franzen[2] has reviewed the history of research on these pathogens. The class or order Microsporidia was elevated to the phylum Microspora by Sprague in 1977.[1] In 1998 Sprague and Becnel suggested that the term "Microsporidia" instead be used for the phylum name.[3] Although the Microsporidia were historically considered "primitive" protozoa, molecular phylogenetic analysis has led to the recognition that these organisms are not primitive, but degenerate, and that they are related to the Fungi, either as a basal branch of the Fungi or as a sister group.[6,7] Molecular phylogeny has also led to the recognition that traditional phylogeny based on structural observations may not reflect the relationships among the various microsporidian species and genera.

The Microsporidia infect almost all animal phyla, including other protists. They are important agricultural parasites in insects, fish, laboratory rodents, rabbits, fur-bearing animals, and primates and are found infecting various animals kept as household pets.[4,5,8] Some species of these pathogens are used as biologic control agents for destructive species of insects.[9] In their hosts the majority of the Microsporidia infect the digestive tract, but infections of almost all organ systems have been documented.[10] Microsporidia were first recognized in mammalian tissue samples more than 75 years ago[11] and described in 1959[12] as being human pathogens when they were found in a child with encephalitis. Many of the human pathogenic Microsporidia are zoonotic or waterborne infections, or both. In the immunosuppressed host (e.g., those treated with immune-suppressive drugs or infected with human immunodeficiency virus [HIV], particularly at advanced stages of the disease), the Microsporidia can produce a wide range of clinical diseases. Reports of diarrheal syndromes associated with microsporidiosis and HIV infection were first reported in 1985,[13] and the number of articles describing human disease increased rapidly after 1990. Since the advent of antiretroviral therapy (ART) the incidence of microsporidiosis has declined in the HIV-infected population. In addition to gastrointestinal (GI) tract involvement, patients with encephalitis, ocular infection, sinusitis, myositis, and disseminated infection are well described in the literature.[4,5] These organisms are also seen in immune-competent individuals.

The phylum Microsporidia (Microspora) contains at least 1500 species distributed into approximately 200 genera, of which the following have been demonstrated in human disease (Table 270.1)[4,5,14]: *Nosema* (*Nosema corneum,* renamed *Vittaforma corneae*[15]; *Nosema algerae,* reclassified initially as *Brachiola algerae*[16] and now as *Anncaliia algerae*[17]); *Pleistophora; Encephalitozoon; Enterocytozoon*[13]; *Septata*[18] (reclassified as *Encephalitozoon*[19]); *Trachipleistophora*[20,21]; *Brachiola*[16]; *Anncaliia*[17]; *Tubulinosema*[22,23]; *Endoreticulatus*[24]; and *Microsporidium.*[4] *Encephalitozoon hellem* has been associated with superficial keratoconjunctivitis, sinusitis, respiratory disease, prostatic abscesses, and disseminated infection.[4,10,25] *Encephalitozoon cuniculi* has been associated with hepatitis, encephalitis, and disseminated disease.[26–28] *Encephalitozoon (Septata) intestinalis* is associated with diarrhea, disseminated infection, and superficial keratoconjunctivitis.[18,29,30] *Nosema, Vittaforma,* and *Microsporidium* have been associated with stromal keratitis associated with trauma in

TABLE 270.1 Microsporidia Identified as Pathogenic to Humans

GENUS AND SPECIES	REPORTED INFECTIONS	ANIMAL HOSTS[a]
Encephalitozoon		
E. cuniculi[b]	Hepatitis, peritonitis, encephalitis,[c] urethritis, prostatitis, nephritis, sinusitis, keratoconjunctivitis, cystitis, diarrhea,[c] cellulitis, disseminated infection	Mammals (rabbits, rodents, carnivores, primates)
E. hellem[b]	Keratoconjunctivitis,[c] sinusitis, pneumonitis, nephritis, prostatitis, urethritis, cystitis, diarrhea, disseminated infection	Psittacine birds (parrots, lovebirds, parakeets), birds (ostrich, hummingbirds, finches)
E. intestinalis[b]	Diarrhea,[c] intestinal perforation, cholangitis, nephritis, keratoconjunctivitis	Mammals (donkeys, dogs, pigs, cows, goats, primates)
Enterocytozoon		
E. bieneusi	Diarrhea,[c] wasting syndrome, cholangitis, rhinitis, bronchitis	Mammals (pigs, primates, cows, dogs, cats), birds (chickens)
Trachipleistophora		
T. hominis[b]	Myositis, keratoconjunctivitis,[c] sinusitis	Unknown
T. anthropopthera[b]	Encephalitis, disseminated infection, keratitis	Unknown
Pleistophora		
P. ronneafiei	Myositis	Unknown
Pleistophora sp.	Myositis[c]	Fish
Anncaliia		
A. vesicularum	Myositis	Unknown
A. algerae[d]	Keratoconjunctivitis, myositis, skin infection	Mosquitoes
A. connori	Disseminated infection	Unknown
Nosema		
N. ocularum	Keratoconjunctivitis[c]	Unknown
Vittaforma		
V. corneae[b]	Keratoconjunctivitis,[c] urinary tract infection	Unknown
Tubulinosema		
T. acridophagus (and *Tubulinosema* sp.)	Myositis, disseminated infection (skin, liver, peritoneum, lung and retinal involvement)	Insects (*Drosophila melanogaster* and grasshoppers)
Endoreticulatus		
Endoreticulatus sp.	Myositis[c]	Lepidopteran insects
Microsporidium		
M. africanus	Corneal ulcer[c]	Unknown
M. ceylonensis	Corneal ulcer[c]	Unknown

[a]Animals in which organism has been found other than humans.
[b]Organism can be grown in tissue culture.
[c]Cases reported in immune-competent hosts.
[d]Previously called *Brachiola* and *Nosema*.[16,17]

immune-competent hosts.[25,31] *Pleistophora, Anncaliia, Tubulinosema, Endoreticulatus,* and *Trachipleistophora* have been associated with myositis and sometimes with associated disseminated disease.[20,23,32–34] *Trachipleistophora* has been associated with encephalitis, keratitis, and disseminated disease.[21,35] *Enterocytozoon bieneusi,* originally described in humans,[13] is associated with malabsorption, diarrhea, and cholangitis.[36,37]

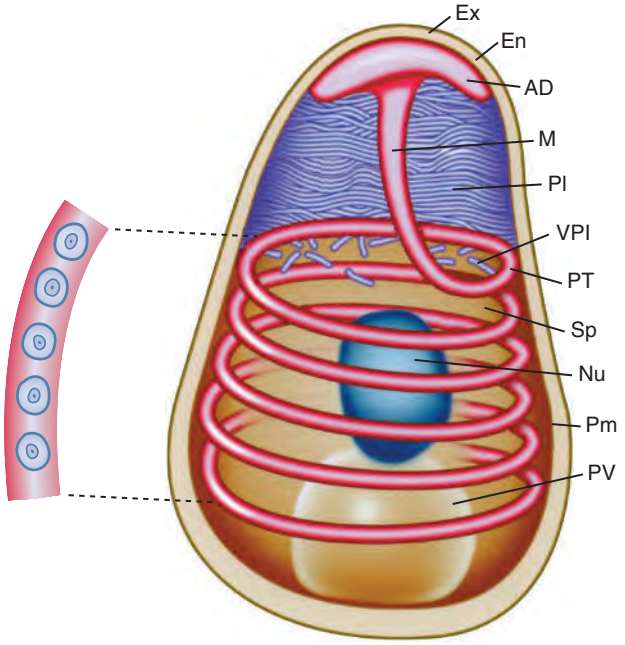

FIG. 270.1 Structure of a microsporidian spore. Depending on the species, the size of the spore can vary from 1–10 μm, and the number of polar tubule coils can vary from a few to 30 or more. Extrusion apparatus consists of the polar tube *(PT),* vesiculotubular polaroplast *(VPl),* lamellar polaroplast *(Pl),* anchoring disk *(AD),* and manubrium *(M).* This organelle is characteristic of the Microsporidia. The inset demonstrates a cross section of the polar tube coils (five coils in this spore), demonstrating the various concentric layers of different electron density and electron-dense core present in such cross sections. The nucleus *(Nu)* may be single, such as in *Encephalitozoon* spp., or a pair of abutted nuclei known as a diplokaryon, such as in *Nosema* spp. The endospore *(En)* is an inner, thicker, electron-lucent region. The exospore *(Ex)* is an outer electron-dense region. The plasma membrane *(Pm)* separates the spore coat from the sporoplasm *(Sp),* which contains ribosomes in a coiled helical array. The posterior vacuole *(PV)* is a membrane-bound structure. (Modified from Wittner M, Weiss LM, eds. *The Microsporidia and Microsporidiosis.* Washington, DC: American Society of Microbiology Press; 1999:199.)

GENERAL CHARACTERISTICS

The Microsporidia are eukaryotes containing a nucleus with a nuclear envelope, an intracytoplasmic membrane system, chromosome separation on mitotic spindles, vesicular Golgi,[38] and a mitochondrial remnant organelle called a mitosome.[39] Microsporidia are ubiquitous in the environment and infect almost all invertebrates and vertebrates.[4,10,14] They form characteristic unicellular spores (Fig. 270.1), which are environmentally resistant. Microsporidian spore size and shape vary depending on the species. Whereas microsporidian spores can be as large as 12 μm, microsporidia infecting humans have spores that range from 1 to 3 μm × 1.5 to 4 μm in size and are usually ovoid. Spore structure is characteristic of the phylum.[40] The spore coat consists of an electron-dense, proteinaceous exospore, an electron-lucent endospore composed of chitin and protein, and an inner membrane or plasmalemma.[41] Proteins making up the spore coat have adhesion domains that may facilitate the binding of microsporidian spores to either the cell surface or mucus of the GI tract before germination.[42] A defining characteristic of all microsporidia is an extrusion apparatus that consists of a polar tube attached to the inside of the anterior end of the spore by an anchoring disk and, depending on the species, forms 4 to approximately 30 coils around the sporoplasm in the spore. Proteomic and genetic studies have defined some of the proteins of the polar tube and spore wall,[43–45] the presence of *O*-mannosylation on these proteins,[46–48] and how these proteins interact.[49]

During germination the polar tube rapidly everts, forming a hollow tube that brings the sporoplasm into intimate contact with the host cell (Fig. 270.2). The polar tube provides a bridge to deliver the sporoplasm to the host cell. The mechanism whereby the polar tube

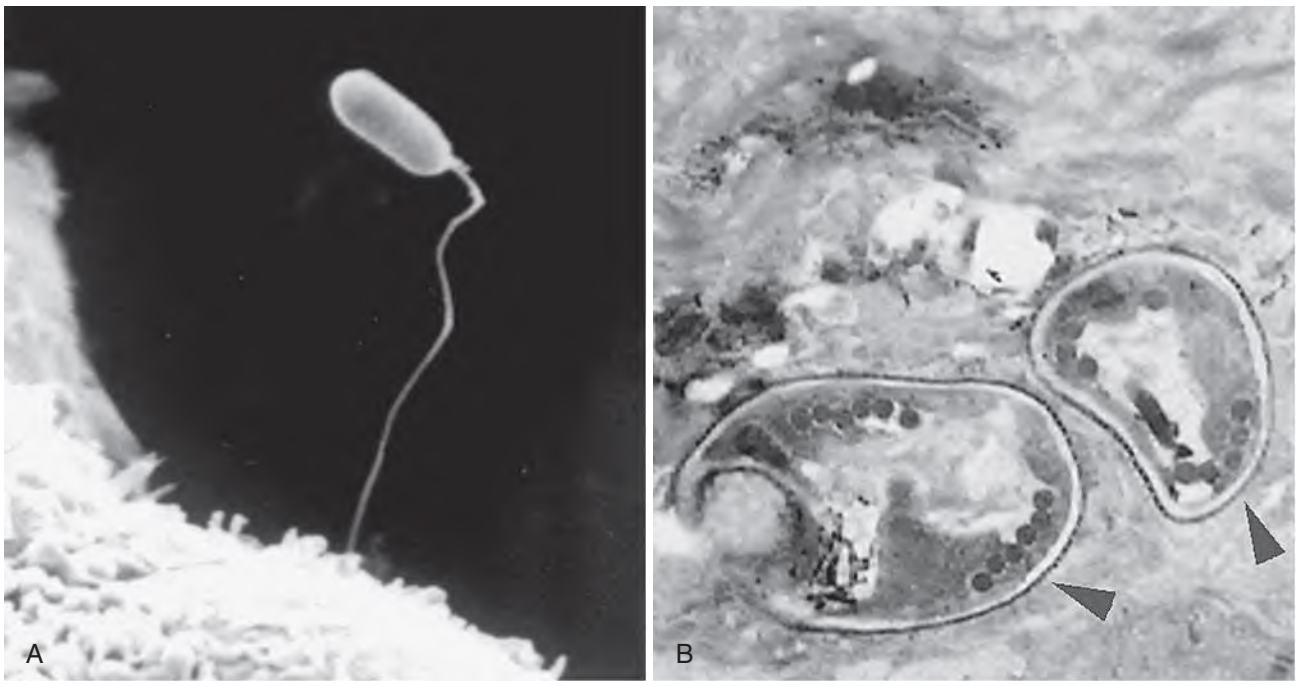

FIG. 270.2 Microsporidian polar tube. (A) Scanning electron micrograph of a tissue culture demonstrating *Encephalitozoon intestinalis* invading a Vero cell in vitro. (B) Transmission electron micrograph of conjunctival scraping demonstrating microsporidia *(Encephalitozoon hellem).* Arrowheads identify thick-walled spores with single nuclei and five or six coils of polar tubes. ([A] from Kock NP. *Diagnosis of Human Pathogenic Microsporidia.* Dissertation, Berhard Nocht Institute for Tropical Medicine, Hamburg, Germany. With permission of N.P. Kock, C. Schmetz, and J. Schottelius.)

interacts with the host cell membrane is not known, but it may require the participation of host cell proteins such as actin.[50] It is possible that the sporoplasm interacts with the host cell membrane as it emerges from the polar tube. If a spore is phagocytosed by a host cell, germination occurs, and the polar tube can pierce the phagocytic vacuole, delivering the sporoplasm into the host cell cytoplasm. The overall process of germination and formation of the polar tube delivers the sporoplasm to the host cell, functioning essentially like a hypodermic needle.[51,52]

The polar tube discharges from the anterior pole of the spore in an explosive reaction, occurring within less than 2 seconds.[51] Conditions that promote germination vary widely among species, presumably reflecting the organisms' adaptation to their host and external environment[53,54] (reviewed by Keohane and Weiss[55]). Conditions that promote spore discharge include pH shifts, dehydration followed by rehydration, various cations and anions, mucin or polyanions, hydrogen peroxide, ultraviolet irradiation, and the calcium ionophore A23187. Inhibitors of spore discharge include magnesium chloride, ammonium chloride, low salt concentrations, sodium fluoride, ultraviolet light, temperatures higher than 40°C, calcium channel antagonists, calmodulin inhibitors, cytochalasin D, demecolcine, and itraconazole. Regardless of the stimuli required for activation, most microsporidia appear to exhibit the same response to the stimuli, an increase in intrasporal osmotic pressure. This results in an influx of water into the spore accompanied by swelling of the polaroplasts and posterior vacuole before spore discharge. In *A. (Nosema) algerae,* it has been proposed that activation brings trehalose in contact with the enzyme trehalase, causing an increase in osmotic pressure.[56,57]

Microsporidia display a number of characteristics that are unusual for eukaryotic organisms. They have prokaryotic-size ribosomes[58] that do not have a 5.8S ribosome subunit but do have sequences homologous to the 5.8S region in the 23S subunit.[59] The small subunit of ribosomal (rRNA) of most of the microsporidia that have been sequenced is significantly shorter than both eukaryotic and prokaryotic small subunit rRNA.[60,61] These rRNA genes are in a subtelomeric location on each chromosome of *E. cuniculi*[62,63] and lack the paromomycin binding site seen in protozoa and animals.[64] The genome size of the Microsporidia varies from 2.3 to 19.5 megabase (Mb).[65] The genomic size of the Encephalitozoonidae is less than 3.0 Mb, making them among the smallest eukaryotic nuclear genomes so far identified. There are almost no introns in the compact genome of *E. cuniculi,* the gene density is high, and proteins are shorter than the corresponding genes in *Saccharomyces cerevisiae.* There appears to be a high degree of gene conservation among the Microsporidia.[66] Genome data for the Microsporidia are available from the Microsporidia database (http://microsporidiadb.org/micro/),[67] which is part of the EuPath database (http://eupathdb.org/eupathdb/) website. This website includes the genomes of the human pathogenic microsporidia *E. cuniculi*[62,68] (all three strains[69]), *E. hellem,*[70] *E. intestinalis,*[71] *E. bieneusi,*[72,73] *A. algerae, V. corneae*, and microsporidia found in other organisms. Chromosomal analysis of *E. cuniculi*[74] and studies on the heterogeneity of gene loci in both *E. cuniculi*[75] and *Nematocida parisii*[76] indicate that microsporidia are diploid. The karyotypes of several members of the phylum Microsporidia have been determined by pulsed-field electrophoresis. Small subunit rRNA (16S rRNA) diverges greatly from the small subunit rRNA sequences of other eukaryotes, and microsporidian rRNA genes are significantly shorter than those of eukaryotes and prokaryotes.[77] Sequence data of rRNA from the microsporidia have been used to develop diagnostic polymerase chain reaction (PCR) primers and to study phylogenetic relationships[78,79] (reviewed by Weiss and Vossbrinck[61]).

Data on rRNA sequences have proven useful in the identification of environmental reservoirs for microsporidia that infect humans. *E. cuniculi* isolates from various animal species have been identified and separated on the basis of the number of tetranucleotide repeats (5′-GTTT-3′) in the intergenic spacer region of their rRNA genes.[80] Differences have also been found in the intergenic spacer region of rRNA genes of *E. bieneusi* and have been used to identify isolates associated with particular animals or environments.[81–84] Currently, all the different isolates that have been identified within an animal species are considered to be the same microsporidian species; however, it is possible that there may be more than one species of *Enterocytozoon* in its mammalian and avian hosts.

PHYLOGENY OF THE MICROSPORIDIA

Microsporidia are currently classified based on their ultrastructural features, including the size and morphology of the spores, number of coils of the polar tube, developmental life cycle, and host-parasite

relationship (see Fig. 270.2).[5] Tuzet and colleagues,[85] Sprague,[1] Larsson,[86] Issi,[87] Weiser,[88] and Sprague and associates[14] have provided overviews of the history, ultrastructural and structural characteristics, and life-cycle differences among taxa of the Microsporidia. Microsporidia can be divided into three main groups:

1. The "primitive" (Metchnikovellidae) hyperparasites of gregarines in annelids may be distinguished from the other microsporidia by the presence of a rudimentary polar filament and a spore without a polaroplast.
2. The Chytridopsidae, Hesseidsae, and Burkeidae may be seen as "intermediates," having a short polar filament and minimal development of the polaroplast and endospore.
3. The "higher" Microsporidia have a well-developed polar filament, polaroplast, and posterior vacuole.

Differences among modern classifications of the Microsporidia focus on the characteristics used to divide the third group, the higher Microsporidia, into subgroups. Molecular analysis of rRNA genes is also useful for classification.[61,89,90] Antigenic differences between the Microsporidia demonstrable by sodium dodecylsulfate polyacrylamide gel electrophoresis and immunoblot analysis have been used as adjunctive evidence when determining phylogenetic relationships among the Microsporidia infecting humans.[19,91]

The general features of the life cycle are as follows:

1. Spores are ingested or inhaled and then germinate, resulting in extension of the polar tube, which injects the sporoplasm into the host cell.
2. Merogony follows, during which the injected sporoplasm develops into meronts (the proliferative stage), which multiply, depending on the species, by binary fission or multiple fission, forming multinucleate plasmodial forms.
3. The next step is sporogony, during which meront cell membranes thicken to form sporonts.
4. After subsequent division, the sporonts give rise to sporoblasts, which go on to form mature spores without additional multiplication. Once a host cell becomes distended with mature spores, the cell ruptures, releasing mature spores into the environment and thereby completing the life cycle.

The combination of multiplication during merogony and sporogony results in a large number of spores being produced from a single infection and illustrates the enormous reproductive potential of these organisms.

Data on rRNA gene sequences (e.g., molecular phylogenetic data) provide additional evidence for the assignment of microsporidia to specific genera and provide a method to distinguish morphologically similar microsporidia at the species level. Analysis of the rRNA genes of a variety of microsporidia highlights the polyphyletic nature of the Microsporidia and brings into doubt the use of any single character for developing higher taxonomic groupings. For example, *E. hellem* and *E. cuniculi* are indistinguishable at the ultrastructural level, and *E. intestinalis* has a distinct extracellular matrix surrounding the sporoblasts and spores; however, on the basis of rDNA analysis, *E. intestinalis* and *E. cuniculi* are more similar to each other than to *E. hellem*.[92]

Molecular phylogenetic data indicate that the Microsporidia are related to the Fungi (either as a basal branch of the Fungi or as a sister group[6,7]) and are not primitive eukaryotes.[93–95] As early as 1994, on the basis of β-tubulin sequence analysis, it was suggested that the Microsporidia were not ancient eukaryotes but instead were related to the Fungi.[96] Keeling and Doolittle[97] reported similar results for α-tubulin from three species of microsporidia *(E. hellem, Nosema locustae, Spraguea lophii)* and for β-tubulin from *E. hellem*. Analysis of the heat shock protein gene *(hsp70)* for *Nosema locustae, Vairimorpha necatrix, E. cuniculi,* and *E. hellem* provided confirmatory evidence of this relationship.[98–101] Comparative analysis of the largest subunit of the RNA polymerase II *(RPB1)* gene produced a phylogeny similar to the result obtained from an analysis of *hsp70* or the β-tubulin gene. Additional evidence for the relationship of the Microsporidia to the Fungi includes the following:

1. The *E. cuniculi* genes for thymidylate synthase and dihydrofolate reductase are separate genes.[102]
2. The small subunit rRNA gene of microsporidia lacks a paromomycin binding site, similar to the fungi.[103]
3. The elongation factor-1α sequence of the microsporidian *Glugea plecoglossi* has an insertion that is found only in fungi and animals, not in protozoa.[103,104]
4. Microsporidia display similarities to the Fungi during mitosis (e.g., closed mitosis and spindle pole bodies[105]) and meiosis.[106]
5. Microsporidia have chitin in their spore wall and store trehalose, as do fungi.
6. Analyses of many genes (e.g., glutamyl-tRNA synthetase, seryl-tRNA synthetase, vacuolar adenosine triphosphate [ATP]ase, TATA box–binding protein, transcription initiation factor IIB, subunit A of vacuolar ATPase, and guanosine triphosphate [GTP]-binding protein and transcription factor IIB sequences[68,107]) support a relationship between the Microsporidia and the Fungi.
7. Analysis of the *E. cuniculi* genome and other microsporidian genomes demonstrates that most microsporidian proteins are most similar to fungal homologues.[6,7,68]
8. The presence in *E. cuniculi* of the principal enzymes for the synthesis and degradation of trehalose confirm that this disaccharide could be the major sugar reserve in microsporidia, as is seen in many fungi.
9. Analysis of glycosylation pathways has indicated that *O*-mannosylation (e.g., *O*-linked glycosylation with mannose), as seen in fungi, occurs in microsporidia. Experimental evidence has confirmed that *O*-mannosylation occurs on the major polar tube protein PTP1.[46]
10. Keeling,[108] in an analysis of β-tubulin data that included additional species of microsporidia and more fungal phyla, found that the Microsporidia were a sister group to the Zygomycota. Comparative analysis using the complete genomes of several microsporidia also supports a similar relationship of the Microsporidia with the Fungi.[6,7]
11. Microsporidian genomes display two syntenic ribosomal genes (*RPL21* and *RPS9*) that are also syntenic throughout the Fungi but are linked in other eukaryotic lineages.
12. An identified microsporidian sex-related locus is highly similar to the sex locus of the Zygomycetes; both contain genes for a triose phosphate transporter (TPT), a high mobility group protein, and an RNA helicase.[7,109,110]

Environmental microbiome studies have identified a previously overlooked large and diverse group of organisms that have been termed Cryptomycota[111] and, although poorly described at the biologic level, are closely related to the Microsporidia. Genome-scale phylogenies place microsporidia together with Cryptomycota as the basal branch of the fungal kingdom or as a sister phylum to the Fungi. Cryptomycota that grow as intranuclear parasites of ameba have been discovered, and these parasites bear strong morphologic similarities to the Microsporidia.[112] These, and other microsporidia-like organisms, such as *Mitosporidium daphniae*, *Nucleophaga terricolae,* and *Paramicrosporidium*, demonstrate that more environmental sampling is required to resolve the relationship of fungi and microsporidia and to provide a better understanding of the Cryptomycota and the origin of microsporidia.

EPIDEMIOLOGY

Microsporidian spores are commonly found in surface water, and human pathogenic microsporidia have been found in municipal water supplies, tertiary sewage effluent, and ground water.[113–118] It is likely that many of the Microsporidia are waterborne pathogens, and they can be transmitted by food.[119] Water contact has been found to be an independent risk factor for microsporidiosis in some studies[120,121] but not in others.[122,123] Outbreaks of *V. corneae* infection have been associated with hot springs exposure[124] and exposure to soil.[125,126] *E. cuniculi* spores remain viable for 6 days when in water and 4 weeks when dry at 22°C, and *Nosema bombycis* spores may remain viable for 10 years in distilled water.[127] Spores may be killed, however, by exposure for 30 minutes to 70% ethanol, 1% formaldehyde, or 2% Lysol or by autoclaving at 120°C for 10 minutes.[128] Most microsporidian infections are transmitted by oral ingestion of spores, with the site of initial infection being the GI tract. Viable infective spores of microsporidia are present in a number of body fluids (e.g., stool, urine, respiratory secretions) during infection,

suggesting that person-to-person transmission can occur and that ocular infection may be transmitted by external autoinoculation caused by contaminated fingers.[129] It has been possible to transmit *E. cuniculi* via rectal infection in rabbits, suggesting the possibility of sexual transmission.[130] *E. hellem* has been demonstrated in the respiratory mucosa and in the prostate and urogenital tract of patients, raising the possibility of respiratory and sexual transmission in humans.[131,132] Person-to-person transmission is supported by concurrent infections in cohabiting homosexual men.[133] Although congenital transmission of *E. cuniculi* has been demonstrated in rabbits, mice, dogs, horses, alpaca, foxes, and squirrel monkeys, no such congenital transmission has been demonstrated in humans.[134]

There have been two clusters of microsporidiosis due to transplanted organs.[135] Three patients were infected by transplanted organs (kidney, kidney, and bilateral lungs) from an *E. cuniculi*–seropositive donor, and kidney biopsy provided the diagnosis in all three cases. Onset of symptoms (fever, renal dysfunction, and encephalopathy) was 7 to 10 weeks posttransplant, and death in one recipient of a kidney may have been directly related to failure of the transplanted organ. The surviving kidney recipient received 6 months of albendazole therapy and remained healthy afterward.[135] In the second cluster, which also involved three patients infected by transplanted organs (kidney, liver, heart/kidney), the patients presented with neurologic symptoms, including headache and encephalitis.[136] Kidney or urine specimens demonstrated *E. cuniculi* in these patients. The liver transplant patient responded to albendazole treatment.[136]

Many of the Microsporidia probably cause zoonotic infections in humans (see Table 270.1).[137] Microsporidia of the genus *Encephalitozoon* are widely distributed parasites of mammals and birds, and the onset of microsporidiosis has been associated with exposure to livestock, fowl, and pets.[138] *E. hellem* infections have been described in lovebirds and budgerigars (parakeets),[8] and *E. hellem* infection has been reported in a patient who had infected lovebirds.[139] Up to 30% of dogs in animal shelters may excrete microsporidia in their stools. Microsporidia of the genus *Encephalitozoon* were found in the stools of many animals in an epidemiologic survey in Mexico.[120] *Enterocytozoon bieneusi* appears to be widely distributed[82–84] and has been reported in many mammals, including pigs,[140] dogs,[141] chickens,[142] pigeons,[143] falcons, and simian immunodeficiency virus (SIV)-infected rhesus monkeys.[144] There is a documented case of transmission of *E. bieneusi* between a child and guinea pigs.[145] Enterocytozoonidae such as *Nucleospora* (previously *Enterocytozoon*) *salmonis* are pathogens found in fish. *Nosema* and *Vittaforma* infections are believed to be caused by traumatic inoculation of environmental spores of insect pathogens into the cornea.[15,31,146] *Tubulinosema* and *Anncaliia* are insect pathogens.

Although initially regarded as rare, microsporidia are now believed to be common enteric pathogens that cause self-limited or asymptomatic infections in normal hosts.[138,147] Cases of microsporidiosis have been identified from all continents except Antarctica.[133,148–154] Surveys of pathogens seen in stool samples in Africa, Asia, South America, and Central America have demonstrated that microsporidia are often found during careful stool examinations. In immune-competent hosts most reported cases of microsporidiosis manifested as self-limited diarrhea. It has been observed that latent infection with shedding of spores is common in immune-competent humans.[155] They have included cases of *E. bieneusi* infections in travelers to and residents of tropical countries[82,114,150,156–163] and of *E. intestinalis* infections in travelers to and residents of tropical countries.[164] In addition, there are several reports from India and Singapore of ocular infection with microsporidia, in particular *V. corneae,* presenting as a keratoconjunctivitis in immune-competent hosts.[165–169] In immune-deficient hosts (e.g., those with acquired immunodeficiency syndrome [AIDS] or who have undergone transplantation), most reported cases have manifested as diarrhea with wasting syndrome and disseminated infection. Infections with *E. bieneusi* have been reported in patients who have undergone kidney, liver, or heart-lung transplantation. *Encephalitozoon* spp. infections have been reported in patients who have undergone kidney, pancreas, liver, or bone marrow transplantation. *Tubulinosema acridophagus* has been reported in a patient with bone marrow transplantation, and *A. algerae* has been found in a patient with lung transplantation.[22,170–182] A case of chronic bilateral keratoconjunctivitis caused by *Encephalitozoon* sp. has been reported in a patient taking prednisone (20 mg/day).[183]

Reported prevalence rates in the 25 studies conducted on patients with HIV infection before the widespread use of combination antiretroviral therapy (cART) (1989–98) varied between 2% and 70%, depending on the symptoms of the population studied and the diagnostic techniques used.[a] These studies suggest that asymptomatic carriage can occur in immunocompromised patients. Coinfection with different microsporidia or other enteric pathogens can occur. There was no overall trend in these prevalence studies with regard to country of origin or other demographic characteristics. When combined, these studies identified 375 *E. bieneusi* infections among 2400 patients with chronic diarrhea, for a prevalence of 15% in this population. More recent studies suggest that, in areas where cART is not widely available, the epidemiology of this disease has not changed and that a similar prevalence rate is present.[82] In addition, studies of the prevalence of *E. bieneusi* in HIV-negative individuals in developing countries have also demonstrated prevalence rates of 2% to 70%, depending on the country and population studied (e.g., children or adults, rural or urban).[82] It is clear that since the institution of cART and its associated immune reconstitution, the prevalence of diarrhea among AIDS patients has decreased, as has the incidence of microsporidiosis.

The aggregate data available confirm that the Microsporidia demonstrate strength of association, coherence, and reproducibility with respect to being causative for a diarrheal syndrome. Further evidence of the association of microsporidia with diarrhea is provided by the usefulness of albendazole in the treatment of microsporidian infection. Therapy with albendazole results in cure of the diarrhea associated with elimination of *E. intestinalis* from the stool of infected patients.[190] Treatment with fumagillin has a similar effect in patients with *E. bieneusi* infection.[191,192]

Serosurveys in humans have demonstrated a high prevalence of antibodies to *E. cuniculi* and *E. hellem,* suggesting that asymptomatic infection may be common.[151,193] Serologic cross-reactivity among microsporidia has been demonstrated by both immunofluorescence[194] and immunoblot testing.[195] Singh and coworkers[196] found positive titers in 6 of 69 healthy adults in England, 38 of 89 Nigerians with tuberculosis, 13 of 70 Malaysians with filariasis, and 33 of 92 Ghanaians with malaria. In another study 14 of 115 travelers returning from the tropics and 0 of 48 nontravelers were seropositive.[197] In a study of HIV-positive men 10 of 30 were seropositive and all had traveled to the tropics.[198] Antibodies to *E. intestinalis* were found in 5% of pregnant French women and 8% of Dutch blood donors.[199] In HIV-positive Czech patients 5.3% were seropositive to *E. cuniculi* and 1.3% to *E. hellem*.[200] In Slovakia 5.1% of slaughterhouse workers were seropositive to *Encephalitozoon* spp.[201] In a survey of blood donors in the United States 5% of donors had antibodies to *E. hellem* PTP1 antigen (L. M. Weiss, unpublished observations). Overall, these studies suggest that exposure to microsporidia is common and that asymptomatic infection may be more common than originally suspected.

IMMUNOLOGY

Infection with *E. cuniculi* in many mammals results in chronic infection with persistently high antibody titers and ongoing inflammation (e.g., persistent encephalitis in rabbits and chronic renal disease and congenital transmission in foxes). In immune-competent murine models of *E. cuniculi* infection, ascites develops and then clears; however, if corticosteroids are administered, the mice redevelop ascites, consistent with latent persistence of microsporidia in these animals.[202,203] Studies on immune-deficient mice have demonstrated that latent infection occurs, allowing relapse, and that albendazole treatment cannot eliminate latency in immune-deficient mice.[204] In mice with severe combined immunodeficiency (SCID) or athymic mice, infection with *E. cuniculi* results in death, with visceral dissemination of the organism and persistent ascites.[205] Adoptive transfer of sensitized syngeneic T-enriched spleen cells protects athymic or SCID mice against lethal *E. cuniculi* infection.[206,207] Transfer of naïve lymphocytes or hyperimmune serum failed

[a]References 10,133,138,148,154,184–189.

to protect or prolong the survival of these mice. Cytokine-activated murine peritoneal macrophages can inhibit the replication of *E. cuniculi* in vitro.[208] This inhibition is probably mediated by nitric oxide; studies have demonstrated that inhibition of nitric oxide synthesis inhibits such killing.[209] Studies using macrophages have shown that reactive nitrogen and oxygen species, and iron sequestration, all contribute to the control of *E. cuniculi* in these cells.[210] Mice deficient in inducible nitric oxide synthase, however, had no change in susceptibility to *E. cuniculi* infection.[211]

Humoral immunity is not sufficient for protection against *E. cuniculi* infection because adoptive transfer of immune B lymphocytes into athymic BALB/c (nu/nu) or SCID mice or passive transfer of hyperimmune serum into athymic mice does not protect these animals from death after infection. Nonetheless, during *E. cuniculi* infection there is a strong antibody response to many components of this organism, and many of these antibodies are cross-reactive with other microsporidia. Maternal antibodies protect newborn rabbits from infection with *E. cuniculi* during the first 2 weeks of life.[212] The in vitro infectivity of microsporidia is reduced by treatment with immune serum and complement,[206] monoclonal antibody (mAb 3B6) to the spore coat,[213] or polyclonal antibodies to polar tube protein-1 (PTP1) (L.M. Weiss, unpublished data). Immunoglobulin M (IgM) antibodies against PTP1 in normal human serum may play a role in preventing infection with *E. cuniculi*.[214] Overall, it is probable that antibodies play a role in limiting infection in the host, although they are not sufficient to prevent mortality or cure infection.

Interferon-γ (IFN-γ) and interleukin-12 (IL-12) are important for protective immunity against a number of intracellular viral, bacterial, and parasitic infections.[215] Based on in vitro observations, it has been suggested that IFN-γ plays an important role in the protective immunity against *E. cuniculi* infection. Both natural killer cells and γδ T cells, which are increased at early stages of infection, are likely important sources of IFN-γ production. Studies with *E. intestinalis* and *E. cuniculi* have demonstrated that IFN-γ knockout mice cannot clear infection.[216] Treatment of *E. cuniculi*–infected mice with neutralizing antibody to IFN-γ or IL-12 results in increased mortality.[211] The importance of IL-12 is illustrated by the fact that lethal infection with *E. cuniculi* occurs in p40 knockout mice, which are unable to produce IL-12 and have impaired $CD8^+$ cell function to this pathogen.[217,218]

The role of individual T-cell subtypes during *E. cuniculi* infection has been evaluated in murine models, mostly in studies of disseminated infection using intraperitoneal infection.[217] Phenotypic analysis of the spleen cells from infected animals has revealed an increase in the $CD8^+$ T-cell population starting at day 10 after infection, with no significant increase in $CD4^+$ T cells. Mice deficient in $CD8^+$ cells succumb to the parasitic challenge, but there was no change in mortality for mice deficient in $CD4^+$ cells. In most cases $CD8^+$ T cells are primed via IL-2–producing $CD4^+$ T cells, although a normal in vivo $CD8^+$ T-cell response in the absence of $CD4^+$ T cells has been described with many viral infections and appears to occur with *E. cuniculi* infection. A normal antigen-specific $CD8^+$ T-cell response to *E. cuniculi* infection has been found to occur in CD4 knockout mice. The protective effect of $CD8^+$ T cells is mediated by their ability to produce cytokines and to reduce the parasite load by killing the infected targets in the host tissue.[219] The major killing mechanism exhibited by $CD8^+$ T cells is via the perforin pathway, and mice lacking the perforin gene die when infected with *E. cuniculi*. These observations suggest that the cytotoxic T-cell response is a key factor in the immune response to *E. cuniculi*–infected mice.

When mice are infected with microsporidia orally, instead of by peritoneal injection, studies have shown that both $CD4^+$ and $CD8^+$ T cells are involved in the protective immune response generated by the GI tract.[220,221] However, the $CD8^+$ αβ population was the most important cellular subset in providing protective immunity.[222] IFN-γ production by dendritic cells (DCs) has been demonstrated to be important for priming the gut intraepithelial lymphocyte response after oral infection with *E. cuniculi*.[222]

DCs play a key role in promoting the T-cell response via recognition of the pattern recognition receptors expressed by these pathogens (e.g., TLR4[223] and TLR2[224] have both been described to be important for the immune response to microsporidiosis). Plasmacytoid DCs have been demonstrated in older mice to downregulate $CD8^+$ T-cell responses by inhibiting the maturation of normal DCs after *E. cuniculi* infection.[225]

There are scant data to confirm the immune response to microsporidia in humans. It is clear that a strong humoral response occurs during infection and that it includes antibodies that react with the spore wall and polar tube. The immunosuppressive states associated with microsporidiosis (e.g., AIDS and transplantation) are those that inhibit cell-mediated immunity. Microsporidiosis is usually seen in HIV-infected patients when there is a profound defect in cell-mediated immunity (e.g., a $CD4^+$ cell count $<100/mm^3$); spontaneous cure of microsporidiosis can be induced by immune reconstitution with cART.[226–228] Overall, these data are consistent with observations on the immunology of the mouse model of microsporidiosis and suggest that restoration of cell-mediated immunity and possibly administration of IFN-γ or IL-12 are useful adjuncts for treating microsporidiosis.

PATHOLOGY AND PATHOGENESIS

Gastrointestinal Tract Infections

Infection of the epithelium of the GI tract (small intestine and biliary epithelium) is the most frequent presentation of microsporidiosis (Fig. 270.3). Greater than 90% of these infections are caused by *E. bieneusi,* with the remainder mostly caused by *E. intestinalis*.[37,40,133,138] Because most of these infections are acquired by ingesting spores, it is likely that other species of microsporidia also cause asymptomatic GI infections. Granulomatous hepatitis caused by *E. cuniculi* is commonly seen in mammals infected with this organism, and granulomatous hepatitis caused by *Encephalitozoon* has been reported in patients with HIV infection.[229] Hepatitis with infection of the biliary system (including the portal triad and gallbladder epithelium) caused by *E. bieneusi* has been seen in SIV-infected *Macaca mulatta* (rhesus macaques).[230] Chronic gallbladder infection with *E. bieneusi* is seen in pigs. *E. bieneusi* and *E. intestinalis* infections of the biliary tract can result in sclerosing cholangitis in AIDS patients.[231,232] These observations suggest that the biliary epithelium may be a reservoir for relapse of *E. bieneusi* and perhaps other microsporidia.

E. bieneusi infection does not produce active enteritis or ulceration, although the infection results in variable degrees of villous blunting and crypt hyperplasia. There is a gradient in parasite burden along the small intestine, with the distal duodenum and proximal jejunum having higher burdens than the proximal duodenum.[231] The organism can be found in the ileum but is rarely found in the colon. On endoscopy scalloping of the valvulae conniventes and villous fusion may be evident.[233] The organism is located on the apical surface of the enterocytes of the small intestine and epithelial cells of the biliary tract and pancreas. Spores are rarely found on the basal surface or in the lamina propria.[234,235] This organism rarely disseminates, unlike the Encephalitozoonidae, which are commonly found in the lamina propria and disseminate to visceral organs. Infection may be associated with increased intraepithelial lymphocytes and epithelial disarray. At the villous tips teardrop-shaped cells can be seen during the process of sloughing, which is characteristic of infection with *E. bieneusi.* Spores are smaller (1×1.5 μm) than those of *Encephalitozoon* spp. (1.2×2.2 μm) and more difficult to find in tissue sections (see Fig. 270.3). Infection is associated with malabsorption because of decreased mucosal surface area and functional immaturity of the villous epithelial cells.

E. bieneusi displays a unique intracellular developmental life cycle. A characteristic feature of the organism is the presence of electron-lucent inclusions with a lamellar structure. These inclusions are closely associated with the nuclear envelope, the endoplasmic reticulum, or both. The earliest intraepithelial stages of the parasite are rounded proliferative cells limited by a typical unit membrane in direct contact with the host cell cytoplasm. Nuclear division is not immediately followed by cytokinesis in these cells, resulting in the production of multinucleate proliferative plasmodia. After the production of multiple nuclei, the parasites form electron-dense disklike structures that cluster in stacks of three to six, eventually forming the coiled portion of the polar tube. When these multinucleated sporagonial plasmodia divide by invagination of the plasmalemma, multiple spores are formed. In mature spores the polar

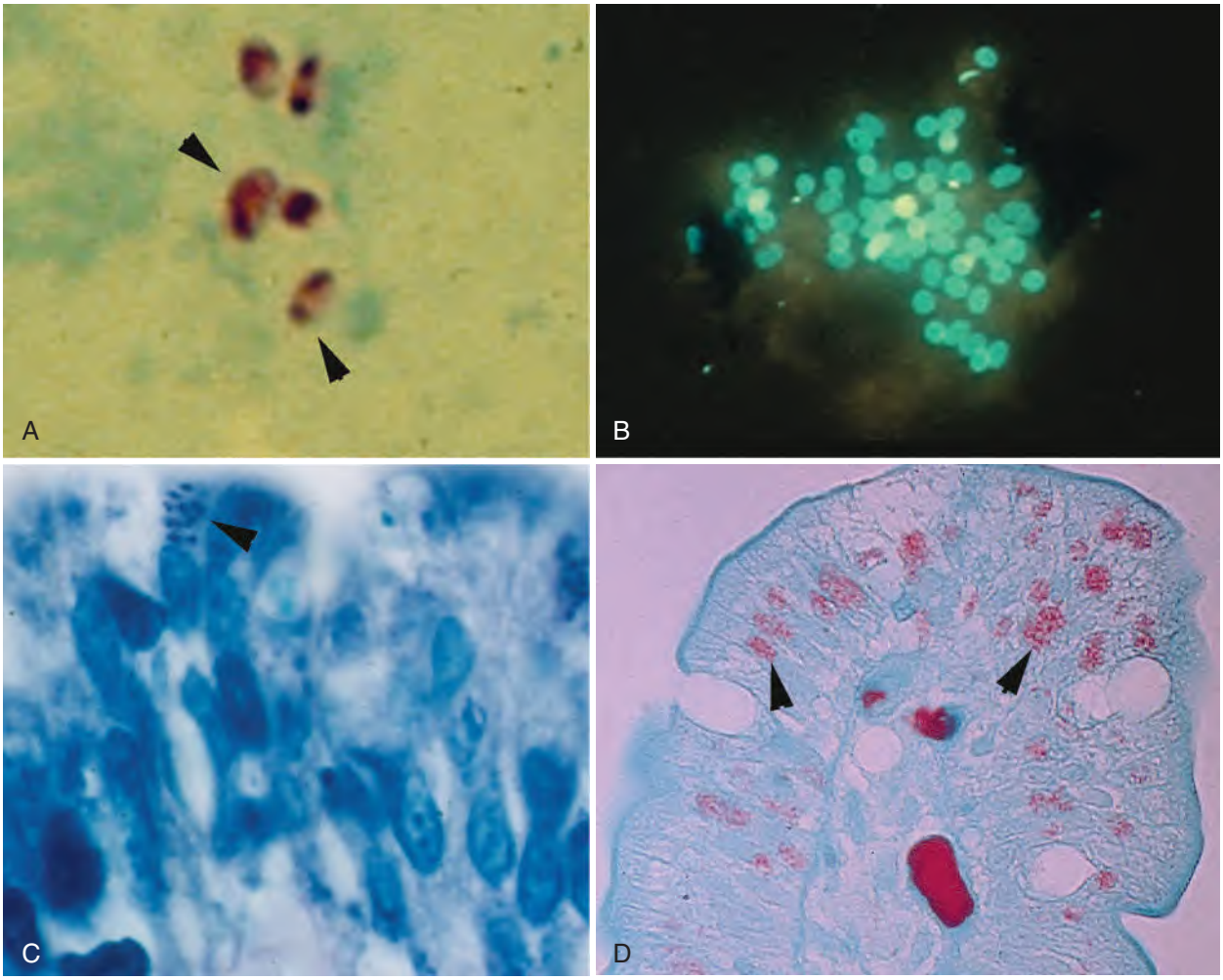

FIG. 270.3 Demonstration of Microsporidia in stool, urine, and tissue samples. (A) Chromotrope 2R stain (modified trichrome stain) of a stool sample from a patient with microsporidian enteritis due to *Enterocytozoon bieneusi*. Arrowheads point to microsporidian spores. Using chromotrope 2R–based stains, spores appear as 1- to 3-µm ovoid light pink structures with a beltlike stripe girding them diagonally and equatorially. (B) Calcofluor white stain (a chemifluorescent optical brightening agent that stains chitin) demonstrating fluorescent *Encephalitozoon hellem* spores in the urine of a patient with disseminated infection. (C) Giemsa stain of a small intestinal biopsy from a patient with electron microscopy–proven *Enterocytozoon bieneusi* enteritis. The arrowhead points to a collection of microsporidian spores at the apical end of an enterocyte. Spores are not found on the basal side or in the lamina propria with this organism. (D) Chromotrope 2R tissue stain of a small intestinal biopsy from a patient with electron microscopy–proven *Encephalitozoon intestinalis* infection. Arrowheads point to microsporidian spores. Note that spores are found in the apical and basal sides of the enterocytes, and in the lamina propria. This is consistent with the ability of this microsporidian to disseminate. *(B courtesy E. Didier, Tulane Regional Primate Research Center, Covington, LA; D courtesy D. Kotler, St. Lukes-Roosevelt Hospital Center and J. M. Orenstein, George Washington University School of Medicine, Washington DC.)*

tubule has five to seven coils that appear in two rows when seen in cross section by transmission electron microscopy.

E. intestinalis is invasive, and spores are commonly found in the apical and basal sides of infected intestinal enterocytes and in cells in the lamina propria, including fibroblasts, endothelial cells, and macrophages (see Fig. 270.3).[236] This pattern of infection is also seen with other *Encephalitozoon* spp. and probably reflects the ability of these infections to disseminate to visceral organs after ingestion. Dissemination can result in necrosis of areas of the bowel, with a presentation resembling that of an acute abdomen[26,237] and perforation of the intestine with peritonitis.[238] Histopathology can demonstrate areas of necrosis and mucosal erosion. In pathology sections the septated parasitophorous vacuole can be seen surrounding the developing spores. *E. intestinalis* spores are easier to detect than *E. bieneusi* spores because of their larger size, strong birefringence, and bluish color on hematoxylin and eosin staining. In addition, spores of *E. intestinalis* are more numerous in tissue. Sporogony is tetrasporous, and tubular appendages originate from the sporont surface and terminate in an enlarged bulblike structure. Unlike infections with other Encephalitozoonidae, *E. intestinalis*–infected cells have a unique parasite-secreted fibrillar network surrounding the developing organisms, so the parasitophorous vacuole appears septate. Mature spores in cross section have a single row of four to seven coils of polar tubules.

E. cuniculi has been described in a case of peritonitis.[239] At autopsy, a mass of omentum was found that displayed focal necrosis, nongranulomatous inflammation, and microsporidian spores. *E. hellem* and *E. cuniculi* have similar developmental life cycles.[38] The genus is characterized by the presence of a phagosome-like parasitophorous vacuole. Nuclei of all stages are unpaired. Meronts divide repeatedly by binary fission. Sporonts divide into two sporoblasts that mature into spores. No tubular appendages or fibrillar networks are produced, as is seen with *E. intestinalis*. In cross section, the mature spore has five to seven coils in single rows.

A case of peritonitis with hepatitis due to *T. acridophagus* infection has been described in a patient with an allogeneic bone marrow transplant.[22]

Genitourinary Tract Infection

Encephalitozoon spp. infect the genitourinary system in most mammals. Contamination of the environment by spores passed in urine is believed

to be the mechanism of transmission of these organisms between successive hosts, which appears to be the case in human infections,[147,190,234,240] in whom infection discovered in any organ (e.g., eye, GI tract, liver, central nervous system [CNS]) is often associated with the shedding of spores in the urine. In HIV-infected patients with keratitis there is usually asymptomatic infection of the urinary tract and bronchial tree. Granulomatous interstitial nephritis composed of plasma cells and lymphocytes is the most frequent pathologic finding. It is associated with tubular necrosis, with the lumen of the tubules containing amorphous granular material. On occasion, microabscesses and granulomas form around necrotic tubules. Spores are located in the necrotic tubes and sloughing tubular epithelial cells. Organisms may also be found in the interstitium. Glomerular involvement is rarely seen. This pattern of interstitial nephritis has been seen in AIDS patients and in those with renal transplants complicated by microsporidiosis.[b] An identical pattern has been described in rabbits with *E. cuniculi* infection.

As spores and infected tubular cells are shed into the bladder, they can infect other epithelial cells of the urogenital tract. Microsporidia have been reported to cause necrotizing ureteritis and cystitis.[234] Inflammatory cystitis has been seen during cystoscopy. These organisms can disseminate from the urogenital epithelial cells and can be found in macrophages, muscle, and supporting fibroblasts of the associated mucosa. Genital tract infection with *Encephalitozoon* has been reported to occur in association with urinary shedding of spores and has included prostatitis with abscess formation.[131] Postmortem examination of eight Cuban women who died of HIV "wasting" syndrome revealed disseminated *E. cuniculi* and *E. hellem* infection with involvement of the fallopian tubes and endometrium.[243] The frequency of microsporidian prostatitis is not known, nor is it known if sexual transmission occurs. A case of urinary tract infection and chronic prostatitis caused by *V. corneae* has also been reported in a patient with AIDS.[146]

Central Nervous System Infection

Granulomatous encephalitis is the classic presentation of *Encephalitozoon cuniculi* infection in many mammals, such as rabbits. This infection has been recognized in a few patients with HIV infection, in whom it "mimicked" toxoplasmic encephalitis.[28] At autopsy, CNS involvement was documented in a case of disseminated *E. cuniculi* (type III or dog strain) involving almost all organs.[244] Spores were present in the cerebral parenchyma, perivascular spaces, and macrophages but were not seen in oligodendrocytes, neurons, astrocytes, or meningeal cells. A second case of *E. cuniculi* encephalitis was caused by the type II or rabbit strain. There have also been case reports of encephalitis caused by *Trachipleistophora anthropopthera*.[21,35] These patients had multiple ring-enhancing lesions on computed tomography (CT) scans suggestive of CNS toxoplasmosis. On pathologic examination the 2- × 2.8-µm birefringent spores were located in the gray matter, and there was extensive necrosis. The disease also involved the heart, kidney, pancreas, thyroid, parathyroid, liver, bone marrow, lymph nodes, and spleen in addition to the brain. The most heavily infected cells were epithelial cells, cardiac myocytes, and astrocytes.

Ocular Infection

Infection with *E. cuniculi, E. hellem,* or *E. intestinalis* can result in punctate keratopathy and conjunctivitis characterized by multiple punctate corneal ulcers (e.g., superficial epithelial keratitis; Fig. 270.4). Microsporidian spores are present in corneal and conjunctival epithelium, which can be obtained by scraping or biopsying the lesions. The organisms do not invade the corneal stroma but remain limited to the epithelium. Inflammatory cells are rarely present. *Trachipleistophora anthropopthera* can also cause keratitis.[245] Ocular disease may be the presenting manifestation when there is disseminated infection with any of the *Encephalitozoon* spp.[91,129,139,229,246] These infections most often occur in the setting of HIV or other immune dysfunction, although there are case reports of superficial epithelial keratitis caused by *Encephalitozoon* spp. in immune-competent patients. Most of these immune-competent cases of *Encephalitozoon* infection have occurred in contact lens wearers. In addition, there are

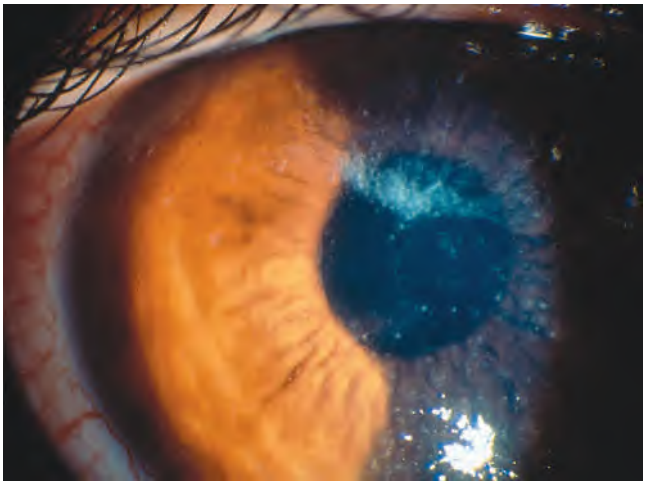

FIG. 270.4 Ocular examination of a patient with keratoconjunctivitis caused by *Encephalitozoon hellem*. Slit-lamp examination findings in a patient with punctate keratoconjunctivitis. A conjunctiva scraping from this patient demonstrated spores consistent with *E. hellem* (see Fig. 270.2B). Sequencing of the small subunit ribosomal RNA gene obtained by the polymerase chain reaction from this conjunctival biopsy confirmed that the infection was caused by *E. hellem*.

numerous reports, involving more than 300 patients from India and Singapore, of *V. corneae* infection presenting as keratoconjunctivitis in immune-competent hosts.[165–169] This microsporidia-associated illness presents as seasonal keratoconjunctivitis and may be more common than previously appreciated. *V. corneae, Microsporidium africanum, Microsporidium ceylonensis*, and *Nosema ocularum* have also been reported to cause infection in other immune-competent patients.[247] These reported infections have usually involved deeper levels of the corneal stroma and have often been associated with trauma. Biopsies have demonstrated necrosis and acute inflammatory cells with some giant cells in several cases. Clinically, these patients have a corneal stromal keratitis and occasionally uveitis. Stromal keratitis caused by a unspeciated microsporidial organism was initially mistaken for corneal graft rejection and only diagnosed in the explanted host button after regrafting.[248]

Musculoskeletal Infection

Myositis with inflammation caused by several microsporidia has been described in humans. The organisms in these case reports have included *Pleistophora ronneafiei, Pleistophora* sp., *Trachipleistophora hominis, Tubulinosema* spp., *Endoreticulatus* spp., *Anncaliia vesicularum* (formerly *Brachiola vesicularum*), and *A. algerae*.[c] A case of endocarditis due to *E. cuniculi* has been described.[249]

The prevalence or incidence of microsporidian myositis in humans is not known. Biopsies from the cases of *Pleistophora* spp. infection have demonstrated atrophic and degenerating muscle fibers infiltrated with focal clusters of large microsporidian spores that were up to 3.4 µm in length. There was a mixed inflammatory response consisting of plasma cells, lymphocytes, eosinophils, and histiocytes that was mild in the two cases occurring in AIDS patients but severe in the case involving an HIV-seronegative person.[33,252,253] *T. hominis* infection was associated with degeneration, atrophy, scarring, and intense inflammation.[20] There are several reports of *Trachipleistophora* spp. causing myositis. The *A. vesicularum* infection, which occurred in a patient with AIDS, was associated with cytolysis around the spores in the muscle fibers, but no cellular immune response was seen.[16] The *A. algerae* infection occurred in a patient with rheumatoid arthritis treated with steroids and monoclonal antibody to tumor necrosis factor-α (TNF-α).[250] In this case there was a minimal cellular response to the numerous spores present in the muscle fibers. The *Tubulinosema* spp. infection occurred in a patient with chronic lymphocytic leukemia, and a Richter transformation

[b]References 172,176,179,181,182,241,242.

[c]References 16,20,23,24,33,34,250,251.

and muscle tissue examination demonstrated abundant clusters of small, ovoid, basophilic organisms that could be stained with Warthin-Starry silver stain.[23] The *Endoreticulatus* spp. infection occurred in an otherwise healthy man who presented with difficulty swallowing and muscle pain.[24] A muscle biopsy demonstrated necrotizing granulomatous inflammation, and a bone marrow biopsy demonstrated increased plasma cells and histiocytes and focal aggregation of microsporidial spores. An examination of urine demonstrated similar microsporidian spores.

Sinus and Respiratory Infection

Respiratory tract involvement is often seen with disseminated infections caused by the Encephalitozoonidae or other microsporidia.[22,254] The pathologic features are nonspecific and may include rhinitis, sinusitis, and nasal polyposis in any combination.[255–261] A tongue ulcer containing *E. cuniculi* spores was reported in a patient with disseminated infection with this organism.[262] In a study of patients presenting with keratitis caused by *E. hellem,* spores of this organism were present in many of these patients' sputum samples in the absence of respiratory symptoms.[263] *E. hellem* infection of the entire length of the respiratory tract, including the terminal bronchioles, associated with erosive tracheitis, bronchitis, and bronchiolitis, has been described in an autopsy report. Spores were seen in the epithelial cells, neutrophils in the bronchiolar wall, cells lining the alveoli, and extracellularly in the alveolar spaces. Case reports have confirmed that *E. cuniculi, E. hellem,* and *E. intestinalis* can cause bronchiolitis with or without pneumonia. Sinus biopsies in AIDS patients with chronic sinusitis and microsporidiosis have demonstrated spores in epithelium and supporting structures.[264] The inflammatory response has been variable, with lymphocytes, neutrophils, macrophages, and occasional granuloma formation. Respiratory tract infection caused by *E. bieneusi* has been reported twice, with spores found in stool, bronchoalveolar lavage (BAL) fluid, and transbronchial biopsy specimens.[265,266] A case of rhinosinusitis caused by *E. bieneusi* has also been reported.[267] It has been suggested that these cases reflect contamination and colonization of the respiratory tract as a result of vomiting rather than dissemination of this organism from the GI tract. In a case of disseminated *T. acridophagus* infection with respiratory failure and pulmonary infiltrates, microsporidian spores were present in BAL fluid.[22]

Skin

Microsporidia infections of the skin have been described. In a child with leukemia the infection was caused by *A. algerae,* with spores infecting the cellular elements of the dermis.[268] In another case an *Encephalitozoon* sp. was reported to be the cause of nodular skin lesions.[269,270] An infection with *T. acridophagus* presented as red macules and papules in a patient with an allogeneic bone marrow transplant.[22] Biopsy demonstrated cysts containing microsporidian spores with minimal inflammatory cell infiltration.

CLINICAL MANIFESTATIONS

The clinical manifestations of microsporidiosis are shown in Table 270.1.

Microsporidian Infection in Non-AIDS Patients

Levaditi and colleagues[271] suggested that microsporidia were associated with human disease as early as 1923, but the first definitive proof of human infection was not reported for another 50 years. In 1973 a 4-month-old athymic male infant died with severe diarrhea and malabsorption. At autopsy the microsporidian *Anncaliia (Nosema) connorii* was discovered in his lungs, stomach, small and large bowel, kidneys, adrenal glands, myocardium, liver, and diaphragm.[272]

In all patients (those with or without HIV infection) the most common symptomatic microsporidian infection is probably diarrhea.[d] *E. bieneusi* has been identified as a cause of self-limited diarrhea in immune-competent hosts, including travelers.[147,161–164,273,274] In epidemiologic studies *E. bieneusi* has been identified in 1% to 10% of African children with diarrhea.[e] Clinical manifestations have included watery, nonbloody diarrhea, nausea, diffuse abdominal pain, and fever.

[d]References 120,133,149,150,156,159,164–171,275.
[e]References 82–84,148,170–182,276–279.

Numerous reports document infection in transplant recipients. The diagnosis is often delayed, perhaps because of the lack of specificity of symptoms and a low index of suspicion in transplant medicine. Kidney biopsy and examination of urine sediment is a frequent modality for diagnosis. Fever and kidney graft dysfunction led to a histologic diagnosis *(E. cuniculi)* in the transplanted organ of two pediatric renal transplant patients.[280] Concomitant urinary tract infection with *E. cuniculi* and *E. bieneusi* was thought to have occurred in a renal transplant recipient, based on PCR of urine sediment, although *E. bieneusi* is rarely found outside the GI and biliary tracts.[281] *E. bieneusi* infection with diarrhea that was successfully treated with fumagillin has been reported in patients with allogeneic hematopoietic stem cell transplantation.[282] Dissemination (spores in urine and BAL) has occurred with *E. cuniculi* in a renal transplant recipient presenting with fever, cough, and acute kidney injury. Treatment with albendazole for 6 months was needed to clear the urinary tract.[283] Disseminated infection with *E. hellem* has been seen in patients with leukemia.[284] Death occurred with *T. acridophagus* infection of liver, skin, and BAL fluid of a patient with diffuse alveolar hemorrhage after allogeneic stem cell transplant for multiple myeloma.[22] Fumagillin was used successfully to treat *E. bieneusi* intestinal disease in pediatric kidney and liver-kidney transplant recipients.[285,286] Diarrhea tends to be self-limited in immune-competent patients but is persistent in patients with immunosuppression. *E. intestinalis* was found in 7.8% of the stools of patients in a survey regarding the causes of diarrhea in Mexico[120] and has been described in travelers with chronic diarrhea. In a study of 98 patients with rheumatic disease treated with anti–TNF-α or other disease-modifying drugs, there was an increased incidence of intestinal microsporidiosis.[287]

Ocular infections with ulcer or deep cornea stroma infection associated with eye pain have also been reported in immune-competent patients. In 1973 and 1981 two cases of corneal microsporidiosis, caused by *Microsporidium africanus* in Botswana[288] and *Microsporidium ceylonensis* in Sri Lanka, were described (*Microsporidium* is used at the generic level for Microsporidia of unknown phylogenetic placement).[289] Additional cases of microsporidian keratitis have been identified in immune-competent hosts.[4,247] One of these organisms was classified as *N. ocularum,*[290] and the other, which was successfully propagated in vitro, was named *N. corneum*[31] (now *V. corneae*[15]). An outbreak of *V. corneae* has been associated with exposure during a rugby tournament in Singapore.[126] *A. algerae* infection of the cornea has also been reported,[268,291] and this infection has been associated with endophthalmitis.[291] Among these immunologically normal patients with corneal infections the outcomes included enucleation, unsuccessful penetrating keratoplasty, successful treatment with a corneal transplant,[246] and therapy with topical agents until keratoplasty.[292] Recently, it has been recognized that seasonal keratitis in both India and Singapore is due to microsporidiosis. More than 300 cases have been reported involving *V. corneae* presenting as keratoconjunctivitis.[165–169] One outbreak was associated with exposure to soil at a sporting event attended by children from several countries. These infections have been treated with various topical agents, including 1% voriconazole,[293] 0.02% polyhexamethylene biguanide,[294] albendazole,[295,296] ciprofloxacin,[295,296] fumagillin,[296] and repeated swabbing.[297] A randomized trial of 0.02% polyhexamethylene biguanide in 145 patients did not demonstrate efficacy for polyhexamethylene compared with no treatment.[294] The majority of these cases of seasonal keratitis resolved spontaneously within 5 days without specific treatment. A case of stromal keratitis due to *T. hominis* has also been reported.[298]

Cerebral infections caused by *E. cuniculi* are commonly described in many animals but have been reported only rarely in immune-competent humans. *Encephalitozoon* infection was demonstrated in a 3-year-old boy with seizures and hepatomegaly by positive IgG and IgM indirect immunofluorescence assays (using *E. cuniculi* as the antigen).[193] Recurrent fever infection with *Encephalitozoon* sp. was also reported in a 9-year-old Japanese boy with headache, vomiting, and spastic convulsions.[12] *E. cuniculi* together with *Streptococcus intermedius* was isolated from a brain abscess in a patient with diabetes.[299]

Pleistophora sp. was identified in the skeletal muscle of an HIV-negative patient and HIV-positive patients with myositis associated with normal creatine phosphokinase (CPK) levels.[33,34,252,253] *A. algerae* infection

of the skin has been seen in a patient with leukemia[268] and in another patient with myositis who had significant elevations in CPK and muscle pain; the latter patient had rheumatoid arthritis treated with steroids and antibody to TNF-α.[250] *A. algerae* has also caused myositis in a patient after a lung transplant,[276] and vocal cord lesions[277] in a patient with chronic lymphocytic leukemia. Five cases of *A. algerae* myositis (four from Australia) have been reported in patients with either rheumatoid arthritis or a lung transplant. The only surviving patient received albendazole.[300] Successful treatment with a combination of fumagillin and albendazole of a case of disseminated infection caused by *A. algerae* in a patient with chronic lymphocytic leukemia has been reported.[301] *E. cuniculi* caused a 4- × 7-cm right atrial vegetation attached to a ventricle pacing lead in an immune-competent male.[249] A recent study found *E. cuniculi* in the periprosthetic tissue of several patients in Poland who presented with hip implant loosening requiring hip replacement, despite an absence of symptoms associated with infection.[302]

Microsporidian Infection in AIDS Patients

Microsporidia were recognized as opportunistic pathogens causing diarrhea and wasting in AIDS patients in 1985.[13] Since then, although most reported cases still involved diarrhea, the spectrum of diseases caused by these organisms has expanded to include keratoconjunctivitis, disseminated disease, hepatitis, myositis, sinusitis, kidney and urogenital infection, ascites, and cholangitis, as well as no symptoms at all.[4,10,37] Postmortem examination revealed microsporidial encephalitis in a patient whose clinical and radiographic presentation had suggested progressive multifocal leukoencephalopathy.[303]

Enterocytozoonidae

GI infection is most common with *E. bieneusi,* and the presentation classically involves chronic diarrhea (which can last years[304]), anorexia, weight loss, and bloating without associated fever. It is most frequently seen in AIDS patients with $CD4^+$ counts lower than 50 cells/μL. Frequent (3–10) bowel movements occur daily, consisting of loose to watery stool that does not contain blood or fecal leukocytes.[10,40,305,306] There is no fever when infection is limited to the intestinal mucosa. Diarrhea is often associated with malabsorption and is worsened by food ingestion. Malabsorption can result in weight loss and a wasting syndrome. The mortality of patients with advanced HIV disease and chronic diarrhea with wasting has been reported to be in excess of 50%.[232] Other intestinal pathogens may occur simultaneously or sequentially with the presence of this or other microsporidia.[307]

Although originally thought to invade only enterocytes, it has been demonstrated that *E. bieneusi* can also invade cholangioepithelium.[232] When present in the cholangioepithelium, this organism has been associated with sclerosing cholangitis, AIDS cholangiopathy, and cholecystitis.[308] Presentations include abdominal pain, nausea, vomiting, and fever; jaundice is rarely seen. Fever is most likely the result of concomitant bacterial biliary infection, which produces the typical clinical manifestations of cholangitis. Imaging studies, including abdominal ultrasonography, CT, endoscopic ultrasonography, and endoscopic retrograde cholangiopancreatography, usually demonstrate dilated biliary ducts, irregularities of the bile duct wall, and gallbladder abnormalities, such as thickening, distention, or the presence of sludge. Papillary stenosis has also been seen. Bilirubin is normal, although most patients have elevated liver function tests (e.g., alkaline phosphatase, γ-glutamyltransferase, aspartate aminotransferase, alanine aminotransferase). Of interest, an *E. bieneusi*–like organism has been identified as a causative factor for cholangitis and hepatitis in SIV-infected rhesus monkeys.[144]

Systemic dissemination is rare with *E. bieneusi.* One case report described this organism in nasal mucosa, which probably resulted from direct inoculation of spores from GI secretions.[309] There are two case reports of respiratory tract involvement with *E. bieneusi* associated with chronic diarrhea, persistent cough, dyspnea, wheezing, and chest radiographs showing interstitial infiltrates.[265,304] Spores of *E. bieneusi* were detected in these cases in stool, BAL fluid, and transbronchial biopsy specimens. *E. bieneusi* has been found to be a cause of proliferative serositis (peritonitis) in macaques *(Macaca mulatta).*

Encephalitozoonidae

Encephalitozoonidae are widely distributed among animals.[310] Three members of the family Encephalitozoonidae have been associated with disease in humans—*E. cuniculi, E. hellem,* and *E. intestinalis* (previously known as *Septata intestinalis*). It appears that these microsporidia have the capacity to disseminate widely in their hosts, and their involvement in most organs has now been documented.[4,26,37,40] The ability of these organisms to disseminate correlates with their ability to grow in many cell types in vivo and in vitro. These organisms have been associated with gastroenteritis, keratitis, sinusitis, bronchiolitis, nephritis, cystitis-ureteritis, urethritis, prostatitis, hepatitis, fulminant hepatic failure, peritonitis, cerebritis, and disseminated infection.[26–28,234,239,244,311] An *Encephalitozoon* sp. has also been reported in a case of nodular skin lesions.[269,270]

The major syndrome associated with microsporidiosis is diarrhea and wasting. This is usually caused by *E. bieneusi* (>90% of cases in the United States) and occasionally *E. intestinalis,* although in Europe this organism may be a more frequent cause of diarrhea.[312] *E. intestinalis* can also cause cholangitis,[18,313] keratoconjunctivitis, osteomyelitis of the mandible,[314] upper respiratory infections, renal failure, keratoconjunctivitis, and disseminated infection in AIDS patients.[190,231,315] Elimination of this parasite by treatment with albendazole correlates with the resolution of symptoms.[316]

E. cuniculi has been associated with hepatitis,[229] peritonitis,[239] hepatic failure,[27] disseminated disease with fever,[244] renal insufficiency, and intractable cough.[317] Granulomatous encephalitis caused by *E. cuniculi* was first described in rabbits in 1922, and cases of encephalitis and seizures caused by *E. cuniculi* have been reported in AIDS patients.[28,244] These infections have been reported to respond to albendazole.[26,317]

E. hellem has been reported to cause disseminated disease associated with renal failure, nephritis, pneumonia, bronchitis, and keratoconjunctivitis.[263,318,319] Punctate keratoconjunctivitis is the most commonly recognized clinical manifestation of infection with this organism. Most of the reports of ocular infection caused by Encephalitozoonidae in the literature have been attributed to *E. hellem,* including three cases originally classified as *E. cuniculi*.[25,320] The remaining cases were caused by *Encephalitozoon* sp. or *E. intestinalis*.[244] Patients present with bilateral coarse punctate epithelial keratopathy and conjunctival inflammation, resulting in redness, foreign-body sensation, photophobia, excessive tearing, blurred vision, and changes in visual acuity. Ocular microsporidian infection in HIV-1–infected patients has been restricted to the superficial epithelium of the cornea and conjunctiva (i.e., superficial keratoconjunctivitis), and it rarely progresses to corneal ulceration (see Fig. 270.4). Physical examination reveals conjunctival hyperemia and superficial punctate keratopathy, without deep corneal ulcers or retinal involvement. Slit-lamp examination usually demonstrates punctate epithelial opacities, granular epithelial cells with irregular fluorescein uptake, conjunctival injection, superficial corneal infiltrates, and a noninflamed anterior chamber. Infection may be bilateral or unilateral. It is often associated with disseminated disease.[262,321,322] Examination of the urine in patients with keratoconjunctivitis often reveals microsporidian spores.[132] This organism is also recognized as an important cause of infection of the nasal epithelium, which in turn causes sinusitis in AIDS patients.[264]

Other Microsporidia

Trachipleistophora hominis is a pansporoblastic microsporidian that has been described in several patients with disseminated disease in the setting of AIDS.[20] It has been reported to cause myositis, sinusitis, and keratoconjunctivitis. *T. anthropopthera* infection presents as encephalitis, myositis, and keratoconjunctivitis.[21,35,245] Several of these patients responded clinically to albendazole. *A. (Brachiola) vesicularum* caused myositis in an HIV-1–infected patient who responded to a regimen of albendazole and itraconazole.[16] Cases of myositis caused by *Pleistophora* sp. and *Pleistophora ronneafiei* have also been reported in patients with AIDS.[33,34,252,323] The myositis presentation of these microsporidia has included myalgias, weakness, elevated serum CPK and aldolase levels, and abnormal electromyograms consistent with inflammatory myopathy. A case of urinary tract infection, prostatitis, and *V. corneae* infection has been reported in an AIDS patient.[146]

DIAGNOSIS

Examination of stool specimens by light microscopy has become the standard method for diagnosing GI microsporidiosis (see Fig. 270.3). Because renal involvement with shedding of spores in the urine is common in all the species of the Microsporidia that disseminate, urine specimens should be obtained whenever the diagnosis of microsporidiosis is considered. This has therapeutic implications because the Microsporidia that disseminate (e.g., *Encephalitozoon* spp.) are usually sensitive to albendazole, whereas those that do not disseminate (e.g., *E. bieneusi*) are resistant. Definitive identification of the Microsporidia causing an infection can be done using ultrastructural examination (e.g., electron microscopy) or molecular techniques (e.g., species-specific PCR assay). If stool examination is negative in the setting of chronic diarrhea (>2 months' duration), endoscopy should be performed. A summary of the available diagnostic tests and their usefulness in patients with suspected microsporidiosis is presented in Table 270.2.

Demonstration of microsporidia by light microscopy is accomplished with staining methods that produce differential contrast between the spores of microsporidia and the cells and debris in clinical samples in which microsporidia are found. Adequate magnification using a 60× to 100× objective is required for visualization because the spores are 1 to 3 µm in size. Chromotrope 2R,[324] calcofluor white (fluorescent brightener 28),[325] and Uvitex 2B[326] are useful selective stains for microsporidia in stool specimens and other body fluids. The chromotrope 2R–based method of Weber and associates[324] is a modification of a standard trichrome stain using a 10-fold higher chromotrope 2R concentration and a longer staining time. The Weber method, modified by Ryan and coworkers[327] (which uses aniline blue in place of fast green) and by Kokoskin and colleagues[328] (which uses a higher temperature), are preferred by some laboratories. With a chromotrope 2R–based stain, the spores appear as 1- to 3-µm ovoid, light pink structures with a beltlike stripe girding them diagonally and equatorially against a green (Weber chromotrope stain) or blue (Ryan modification) background (see Fig. 270.3A). A rapid (11-minute) stain, the Gram-chromotrope stain, combines chromotrope 2R staining with a Gram-staining step and results in violet-staining spores.[329] Microsporidian spores can also be visualized by ultraviolet microscopy using chemifluorescent optical brightening agents, such as calcofluor white M2R (fluorescent brightener 28; Fungi-Fluor; Polysciences, Warrington, PA) or Uvitex 2B (Fungiqual A; Medical Diagnostics, Kandern, Germany), which stain chitin in the spore wall (endospore layer) (see Fig. 270.3B). Such stains also stain fungi and other fecal elements; however, microsporidian spores can be distinguished from yeast as they have a uniformly oval shape and are nonbudding.

In a study that examined 50 electron microscopy-proven microsporidia-positive stool specimens, both the chromotrope 2R and chemifluorescent brightening stains identified 100% of specimens if at least 50 100×-objective fields were examined.[330] With Uvitex 2B, all the 186 stool samples examined from 19 patients with biopsy-proven *E. bieneusi* infection were positive, whereas none of the 55 stool samples from 16 biopsy-negative patients were positive.[326] In another study evaluating Uvitex 2B staining, microsporidia were identified in all the samples known to be chromotrope 2R stain positive and in seven additional samples that were chromotrope negative on initial examination.[331] On reexamination, however, these 7 stool samples were found also to be positive with the chromotrope 2R stain. All patients with positive duodenal biopsies had positive stool examinations according to the chromotrope or chemifluorescent methods. The limit of detecting microsporidia by these techniques appears to be 50,000 organisms/mL. Overall, the sensitivity of the chemifluorescent brightener-based stains is slightly higher than chromotrope-based stains, especially when low numbers of spores are present in a sample; however, the specificity of the chemifluorescent stains is lower (90% vs. 100% in one study). Neither the chromotrope nor the chemifluorescent stain provides information on the species of microsporidia being identified. Although it has been reported that microsporidian spores in food can give a false-positive result, and despite the fact that microsporidia are common in the environment, it does not appear to be a common problem when using stool specimens for diagnosing these infections.

Microsporidia in body fluids other than stool (e.g., urine, cerebrospinal fluid [CSF], bile, duodenal aspirates, BAL fluid, sputum) have been visualized using chemifluorescent optical brightening agents or chromotrope 2R, Giemsa, Brown-Hopps Gram, acid-fast, or Warthin-Starry silver stain.[40,332,333] In general, it is easier to identify microsporidian spores in body fluids other than in stool because of the absence of bacteria and debris, which can be confused with microsporidian spores. Because microsporidian infections usually involve mucosa or epithelium, cytologic preparations are especially useful for diagnosis.[40] Specimens that have been useful for diagnosing microsporidian infections include intestinal and biliary epithelium, epithelium of the cornea and conjunctivae, epithelium of the sinonasal and tracheobronchial regions, renal tubular epithelium, and urothelium. Diagnosis has also been accomplished by examining touch preparations of biopsy material. Microscopic examination of corneal tissue in patients with microsporidian keratitis, obtained by gently rubbing the conjunctiva and cornea with a tissue swab, usually reveals multiple, gram-positive, oval organisms in epithelial cells.

Histologically, microsporidian spores are easily discernible with a modified tissue chromotrope 2R, tissue Gram stain (Brown-Hopps or

TABLE 270.2 Diagnostic Tests for Microsporidiosis

TEST	SPECIMENS	USEFULNESS
Chromotrope or chemifluorescent stain	Urine	This is often positive in cases of disseminated microsporidiosis (e.g., *Encephalitozoon* spp.). It should be done in all suspected microsporidia cases.
	Stool	At least three stools should be examined. The combination of chromotrope and chemifluorescence stains provides the highest sensitivity and specificity. Monoclonal antibodies for immunofluorescent antigen detection are also useful.
	Conjunctival scrapings	This is useful for the diagnosis of keratoconjunctivitis. Urine should also be examined in suspected cases to screen for disseminated microsporidiosis.
	Nasal scrapings	This can be useful for the diagnosis of microsporidian sinusitis. Because most microsporidia associated with sinusitis are present in the kidneys, examination of urine should be routine for suspected sinusitis cases. If these tests are negative, biopsy of the nasal mucosa may be useful for diagnosis.
Endoscopy	Touch preparations	Touch preparations are useful for rapid diagnosis (within 24 hours).
	Biliary fluid	Examination is useful for diagnosis of microsporidian cholangitis.
	Biopsy (small intestine)	Biopsy should be considered for all patients with chronic diarrhea longer than 2-mo duration and negative stool and urine examinations. In this group endoscopy has yielded a diagnosis of microsporidia in up to 30% of patients. Tissue can be examined with chromotrope 2R, tissue Gram, or silver stain. If microsporidia are demonstrated to invade the lamina propria, urine examination should be repeated because *Encephalitozoon* spp. are the most likely causative agents. In this setting albendazole has high treatment efficacy.
Polymerase chain reaction (PCR)	Urine, stool, or tissue	Available from reference laboratories (e.g., Centers for Disease Control and Prevention). PCR allows species identification.
Electron microscopy	Tissue	It provides species identification; it is crucial for identifying new species or for the characterization of microsporidia in unusual or new locations.
Serology	Serum	Not useful for diagnosis but may be useful for epidemiologic surveys.

Brown-Brenn), or Luna stain[334] in sections prepared from tissue fixed using routine procedures. Most microsporidian spores are gram positive in tissue sections. With experience, microsporidia can also be seen on hematoxylin and eosin–stained sections. Other stains that may be useful include periodic acid–Schiff, Giemsa, and Steiner silver stains. Some microsporidia are also acid-fast stain positive. Fresh tissue can also be examined by phase-contrast microscopy; because of their thick wall, unstained spores are retractile, appearing green, and such spores can be birefringent. If possible, biopsy or autopsy material should also be placed in electron microscopy fixative when microsporidiosis is suspected because the definitive diagnosis of species requires ultrastructural information. It is possible, however, to use formalin-fixed tissue for ultrastructural analysis. Molecular methods can also be used on formalin-fixed tissue, although unfixed tissue or tissue fixed in ethanol yields the best results with PCR techniques. The kidney is a frequent site of disseminated disease in transplant recipients, both renal and other organs. Pathologic examination of a renal biopsy in a lung transplant recipient revealed granulomatous interstitial nephritis on hematoxylin and eosin staining, associated with *E. cuniculi* infection. A "beltlike stripe" identified in tissue was accentuated in organisms seen in urine by staining with Gram-chromotrope and a modified trichrome stain. Electron microscopy and species-specific PCR were confirmatory.[335]

Polyclonal serum prepared to *E. cuniculi* has been reported to react with *E. bieneusi*.[195,336] Monoclonal antibodies to *E. hellem,*[337] *E. intestinalis,*[338] and *E. bieneusi*[339–342] have been described. These methods have been used to detect microsporidia in tissue sections using immunofluorescence techniques. Several of these antibodies have been demonstrated to be useful for the examination of stool specimens and have demonstrated good sensitivity and specificity. Detection kits for microsporidia in stool and environmental samples using antibodies to Encephalitozoonidae and *Enterocytozoon bieneusi* are now commercially available (Waterborne, New Orleans, LA).

V. corneae,[31] *E. cuniculi, E. hellem,*[91] *T. hominis,*[20] *T. anthropopthera,*[245] and *E. intestinalis*[30] have been cultivated in tissue culture systems in vitro (for a review, see Visvesvara[343]). *E. bieneusi* has not been cultivated continuously in vitro, although limited in vitro cultivation of *E. bieneusi* has been reported (Dr. S. Tzipori, personal communication, 2006).[344] Adenovirus can mimic the cytopathologic effect of microsporidia.[345] Experimental infection of SIV-infected rhesus monkeys with *E. bieneusi* from human tissue has been demonstrated,[346] and serial propagation has been described in immunocompromised rodents.[347] The isolation of microsporidia from clinical specimens is not a routine procedure and is available in only a few specialized research laboratories.

Serologic tests for diagnosing microsporidiosis have been developed and used for epidemiologic studies. Such serologic tests have, for the most part, not been proven useful for diagnosing microsporidiosis. In a study of 12 AIDS patients with *E. bieneusi,* 2 AIDS patients with *E. intestinalis,* and 2 immune-competent patients with *V. corneae,* enzyme-linked immunosorbent assay titers for *E. hellem, E. cuniculi,* or *V. corneae* were not useful for diagnosis.[348] False-negative titers were present in 7 of the patients with microsporidiosis, and half of the control patients (without clinical microsporidiosis) had positive serology to microsporidia. This is consistent with other AIDS-associated infections in which serology has not proven useful.

A number of molecular diagnostic tests have been developed for pathogenic microsporidia. Weiss and Vossbrinck[65] and Ghosh and Weiss[349] have reviewed the molecular diagnostic tests for microsporidiosis. Homology cloning of the rRNA genes of many of the Microsporidia pathogenic in humans has been accomplished using PCR techniques, and numerous microsporidian rRNA sequences are now in the GenBank database. It has been possible to design PCR primers for these small subunit rRNA genes to identify microsporidia at the species level in clinical samples without the need for ultrastructural examination. Two main approaches have been used for constructing PCR primers for the Microsporidia: the use of universal pan-Microsporidia primers and of species-specific primer pairs. These PCR techniques have been applied to biopsy specimens, urine, cultures, and, more recently, stool specimens, and should greatly facilitate diagnostic and epidemiologic studies.[79,247,349–352] Currently, these molecular tests are available in reference laboratories such as the Centers for Disease Control and Prevention.

THERAPY

Treatment for microsporidiosis is outlined in Table 270.3.

Gastrointestinal and Systemic Disease

Microsporidian infection often occurs in immunocompromised hosts, particularly in those with HIV infection and $CD4^+$ cell counts lower than 50/μL. Clinical studies have demonstrated that improved immune function can result in the clinical response of patients with GI microsporidiosis, with elimination of the organism and normalization of the intestinal architecture.[227,228,353–355] Relapse has been reported in patients who developed failure of their antiretroviral therapy associated with a decline in immune function and falling $CD4^+$ counts. In immunodeficient mice albendazole treatment has not been able to eliminate latent microsporidiosis, suggesting that persistence will occur despite drug treatment in the setting of immune suppression. Overall, these observations suggest that part of the primary treatment of microsporidiosis in the setting of AIDS is the institution of effective cART. There have been limited reports of immune reconstitution syndromes with cART and microsporidiosis.[356]

Although several species of the Microsporidia that infect humans can be grown in vitro (but not without a host cell monolayer), the most common human pathogenic microsporidian, *E. bieneusi,* has yet to be grown continuously in vitro. This has limited in vitro testing of antimicrosporidial agents to those active against *Encephalitozoon* spp. and *V. corneae*.[15,357,358] Several agents have also been evaluated in animal models of microsporidiosis, but until recently no practical animal model had been developed for in vivo studies of *E. bieneusi*.[347] See Costa and Weiss[359] for a review of drugs used against microsporidiosis in humans and animals.

Among the compounds tested in vitro and in vivo for treatment of microsporidiosis, fumagillin and albendazole have demonstrated the most consistent activity and have been demonstrated to have clinical efficacy in human infections with various microsporidia.[f] Albendazole binds to β-tubulin and is active against all the Encephalitozoonidae *(E. hellem, E. cuniculi, E. intestinalis)* in vitro at concentrations lower than 0.1 mg/mL; it is also active in animal models of microsporidiosis.[360] Data on *Encephalitozoon* β-tubulin genes have demonstrated an amino-acid sequence associated with sensitivity to benzimidazoles,[361] whereas the sequences of *Enterocytozoon*[362] and *Vittaforma*[363] demonstrate amino acids associated with albendazole resistance. This is consistent with the observed clinical responses to albendazole in these microsporidia.

Albendazole is 70% protein bound. It is distributed to blood, bile, and CSF and eliminated by the kidneys. Peak serum levels 2 hours after an oral dose are 0.20 to 0.94 μg/mL. Drug absorption is increased if albendazole is taken with food containing relatively high concentrations of fat. After oral administration the hepatic metabolism converts albendazole to albendazole sulfoxide, which is detectable in the systemic circulation. Albendazole is not carcinogenic or mutagenic, although in rats and rabbits at dosages of 30 mg/kg it is embryotoxic and teratogenic. Albendazole is therefore not recommended for use in pregnant women. Side effects are rare, although the following have been reported: hypersensitivity (rash, pruritus, fever), neutropenia (reversible), CNS effects (dizziness, headache), alopecia, GI disturbances (abdominal pain, diarrhea, nausea, vomiting), hair loss (reversible), and elevated hepatic enzyme levels (reversible). There is a report of pseudomembranous colitis after albendazole treatment.[364]

There are few placebo-controlled comparative treatment trials of microsporidiosis caused by *Encephalitozoon* spp., but there are numerous case reports demonstrating the efficacy of 2 to 4 weeks of albendazole 400 mg twice daily for these infections. In a double-blind, placebo-controlled trial of eight patients with AIDS and diarrhea caused by *E. intestinalis,* treatment with albendazole (400 mg twice daily for 3 weeks) resulted in resolution of the diarrhea and elimination of the organism in all eight patients, similar to that seen in several case reports.[g] In case reports of chronic sinusitis, respiratory infection, and disseminated infection caused by *E. hellem,* treatment with 400 mg of albendazole twice daily resulted in resolution of symptoms and clearance of the

[f]References 191,192,256,258,315,360–370.
[g]References 190,240,258,315,316,371,372.

TABLE 270.3 Current Treatment Options for Microsporidiosis

ORGANISM	DRUG	DOSAGE AND DURATION[a]
All microsporidian infections	cART with immune restoration (an increase of $CD4^+$ count to >100 cells/μL) is associated with resolution of symptoms of enteric microsporidiosis. All patients should be offered cART as part of the initial management of microsporidial infection. Severe dehydration, malnutrition, and wasting should be managed by fluid support and nutritional supplement. Antimotility agents can be used for diarrhea control if required.	
Enterocytozoon bieneusi	No effective commercial treatment. Fumagillin (oral) has been effective in a clinical trial. *Alternatives:* Albendazole[a] resulted in clinical improvement in up to 50% of patients in some studies, but was not effective in other studies. Nitazoxanide, 1000 mg bid with food for 60 days, has been used but is less effective in patients with low CD4 counts.	20 mg tid (e.g., 60 mg/day)
Encephalitozoonidae infection (e.g., systemic, sinusitis, encephalitis, hepatitis)		
E. cuniculi	Albendazole	400 mg bid[b]
E. hellem	Albendazole	400 mg bid
E. intestinalis	Albendazole	400 mg bid
Encephalitozoonidae keratoconjunctivitis	Fumagillin solution[c] (70 μg/mL). Patients may also need albendazole[a] if systemic infection is present.	2 drops every 2 hr for 4 days, then 2 drops 4 times a day[d]
Trachipleistophora hominis	Albendazole	400 mg bid
Anncaliia (formerly *Brachiola*) *vesicularum*	Albendazole	400 mg bid
	±Itraconozole	400 mg qd
Tubulinosema (*T. acridophagus* and *Tubulinosema* sp.)	There was no response to albendazole (400 mg/day) in the published cases. Oral fumagillin 20 mg tid would be a reasonable choice.	
Endoreticulatus sp.	Albendazole	400 mg bid

[a]Albendazole, 400 mg bid.
[b]The duration of treatment for microsporidiosis has not been established. Relapse of infection has occurred on stopping treatment. Patients should be maintained on treatment for at least 4 weeks, and most patients should continue on treatment until their CD4 count is higher than 200 cells/μL for at least 6 months after the initiation of cART.
[c]Fumidil B (fumagillin bicylohexylammonium; Mid-Continent Agrimarketing, Overland Park, KS) is used at 3 mg/mL in saline (final concentration of fumagillin, 70 μg/mL).
[d]Eye drops should be continued indefinitely; relapse is common on stopping treatment.
bid, Twice daily; *cART,* combination antiretroviral therapy; *tid,* three times daily; *qd,* daily.
Modified from Costa S, Weiss LM. Drug treatment of microsporidiosis. Drug Resist. *2000;3:1–16.*

organism.[365,366] Clinical improvement was demonstrated with albendazole treatment in a patient with disseminated *E. cuniculi* infection involving the CNS, conjunctiva, sinuses, kidneys, and lungs.[28] It has also been reported to be effective in cases of urethritis,[367] renal failure,[368] and disseminated infection.[317] In addition to its efficacy in *Encephalitozoon* spp., disseminated infections with other microsporidia have also been reported to respond to albendazole treatment. In patients with disseminated infection accompanied by myositis due to *T. hominis* and in a patient with myositis caused by *A. vesicularum,* albendazole (400 mg twice daily) resulted in clinical improvement.[16,20]

In contrast to its success in treating patients with the species of microsporidia that disseminate, albendazole has displayed only limited efficacy against *E. bieneusi* infection. In two studies examining 66 patients with diarrhea caused by *E. bieneusi* during the pre-ART era, symptoms were alleviated in about 50% of patients treated with albendazole, but the presence of *E. bieneusi* persisted during treatment in all patients, and there was no improvement in any patient's D-xylose absorption test.[369,370,373] The symptoms rapidly recurred on discontinuation of albendazole therapy in the patients who had reported symptom alleviation with it. Most other studies have found that albendazole has no efficacy against *E. bieneusi* infection.[374]

Despite a few case reports indicating that metronidazole was effective for *E. bieneusi* intestinal infection, most studies have demonstrated that this drug is not effective against this infection.[240,375,376] In vitro studies have also demonstrated that metronidazole has no activity against microsporidia (e.g., *E. cuniculi*).[357] Other medications used without success to treat GI microsporidiosis are azithromycin, paromomycin (microsporidia lack the rRNA binding site for this drug), and quinacrine. Atovaquone has been anecdotally reported to have limited efficacy in patients with microsporidiosis,[375,377] although it has no in vitro activity.[357] Transient clinical remission has been reported with furazolidone or nitazoxanide (1000 mg twice daily) treatment.[235,378] Anecdotal experience in other immune-suppressed hosts has demonstrated that nitazoxanide can be effective for *E. bieneusi* infection; however, it has had variable efficacy in patients with advanced AIDS and low CD4 counts. Sparfloxacin and chloroquine have demonstrated in vitro activity against microsporidia but have not been used clinically. Prophylaxis with trimethoprim-sulfamethoxazole is not effective for preventing microsporidiosis, and this drug has no in vitro or in vivo activity against these organisms.[379] Thalidomide and octreotide have both been reported to decrease diarrhea in about 50% of patients with microsporidiosis, which is probably secondary to the effect of these agents on the physiology of enterocytes.[380] Examination of the biopsies of patients treated with thalidomide (100 mg daily for 1 month) demonstrated persistence of the parasite and no change in parasite load.

Fumagillin was isolated from *Aspergillus fumigatus* in 1949 and, because of its efficacy against *Entamoeba histolytica* in vitro, it was used during the 1950s to treat amebiasis. Fumagillin is used commercially to treat honeybees infected with the microsporidian *Nosema apis* and has been used to treat infections by microsporidia and myxosporeans in various types of fish.[381,382] Fumagillin and its semisynthetic analogue TNP-470 have been found to have activity in vitro and in vivo against the Microsporidia pathogenic for humans, including *E. cuniculi, E. hellem, E. intestinalis, V. corneae,* and *E. bieneusi*.[h] A dose escalation trial of fumagillin performed on AIDS patients infected with *E. bieneusi* used dosages of 10 mg/day for 14 days, 20 mg/day for 14 days, 40 mg/day for 14 days, and 60 mg/day for 14 days. Altogether, 21 of 29 patients exhibited transient clearing of parasites from their stool; all these patients were in the first three dosage groups. In the 60-mg/day group, 8 of 11 patients did not have spores in their stools at week 6 and remained free of spores in stool specimens for a mean of 11 months. Duodenal biopsies on the same 8 patients did not demonstrate microsporidia by light or electron microscopy. A subsequent randomized trial evaluating 12 patients (with either AIDS or transplants) confirmed that 60 mg/day (given as 20 mg three times daily) effectively treated *E. bieneusi* intestinal infection.[191] Fumagillin has also been successful in treating renal transplant patients with *E. bieneusi* intestinal infection,[181] a

[h]References 191,192,360,375,384–386.

pediatric patient with liver transplantation and *E. bieneusi* infection,[286] and patients with allogenic hematopoietic stem cell transplants with *E. bieneusi* infection.[282] Treatment was associated with resolution of diarrhea, clearance of spores, improvement of Karnofsky scores, and improvement in D-xylose absorption tests. The main limiting toxicity of this treatment was thrombocytopenia, which was reversible on stopping fumagillin treatment. A case of aseptic meningitis due to fumagillin treatment has been described in a patient with a kidney transplant.[383]

Fumagillin, ovalicin, and their analogues (e.g., TNP-470) bind in a selective, covalent fashion to the metalloprotease methionine aminopeptidase type 2 (MetAP2). Methionine aminopeptidase activity is essential for eukaryotic cell survival because removal of the terminal methionine of a protein is often essential for its function and posttranslational modification. Homology PCR assay has been used to demonstrate the presence of *MetAP2* genes in several microsporidia.[387] The crystal structure of *E. cuniculi* MetAP2 has been determined (Molecular Modeling Database [MMDB] ID: 63862; Protein Data Bank [PDB] ID: 3CMK).[388] Data from the *E. cuniculi* genome project[68] indicates that *E. cuniculi* does not have a methionine aminopeptidase type 1 gene *(MetAP1),* unlike mammalian cells, which have both *MetAP1* and *MetAP2;* therefore MetAP2 is an essential enzyme in microsporidia.

Ocular Disease

Solutions of the soluble salt fumagillin bicyclohexylammonium (Fumidil B; Mid-Continent Agrimarketing, Overland Park, KS) applied topically have been demonstrated to be nontoxic to the cornea. Treatment of ocular microsporidiosis can be accomplished using a 3-mg/mL solution of Fumidil B in saline (fumagillin, 70 μg/mL)[384,389–392]; the treatment should be continued indefinitely because recurrence has been reported on stopping these drops. Although clearance of microsporidia from the eye can be demonstrated, the organism is still often present systemically and can be demonstrated in the urine or in nasal smears. In such cases the use of albendazole as a systemic agent is reasonable and effective. Topical treatment with thiabendazole (0.4% suspension), a related benzimidazole, was ineffective in one case of keratitis caused by *E. hellem.* Two patients with *Encephalitozoon*-like organisms were reported to respond to imidazole (fluconazole and itraconazole) administration.[393] Yee and associates[139] described complete improvement with oral itraconazole (200 mg twice daily) in a patient with *E. hellem* infection over a 6-week period after debulking the cornea. However, Diesenhouse and coworkers[384] observed no improvement in a patient with *E. hellem* treated with itraconazole, 100 mg three times daily. In vitro data have not confirmed antimicrosporidial activity for imidazole compounds. Sulfa drugs have had variable results in vitro and in vivo and are not recommended for treatment. Polymyxin B, propamidine isethionate 0.1% (Brolene), gramicidin, neomycin sulfate, and tetracycline appear to have limited efficacy for the treatment of microsporidian infection and should not be used except to treat secondary bacterial infections. Keratoplasty appears to provide temporary improvement in some cases, but no advantage to débridement was found in a series of 120 patients with superficial keratoconjuctivitis.[394] Steroids may be useful for decreasing the associated inflammatory response but have no direct action on microsporidia. *V. corneae* has been described to cause seasonal keratoconjunctivitis.[165–169] These infections have been treated with various topical agents, including 1% voriconazole,[293] 0.02% polyhexamethylene biguanide,[294] albendazole,[295,296] ciprofloxacin,[295,296] and fumagillin[296]; however, a randomized trial of 0.02% polyhexamethylene biguanide in 145 patients did not demonstrate efficacy for polyhexamethylene compared with no treatment.[294] A study from Thailand indicated that topical moxifloxicin was effective for microsporidian keratoconjunctivitis (due to *V. corneae*).[395]

Prevention

There are limited data on effective preventive strategies for microsporidiosis. Currently, no prophylactic agents have been identified for these organisms. Patients have developed microsporidiosis while on trimethoprim-sulfamethoxazole prophylaxis,[379] and microsporidiosis has occurred in patients receiving dapsone, pyrimethamine, itraconazole, azithromycin, and atovaquone.[226] No studies have evaluated albendazole for prophylaxis, but given its relative lack of efficacy for *E. bieneusi* infections, it is unlikely to be effective in preventing most cases of intestinal microsporidiosis. The most effective prophylaxis is the restoration of immune function in immunocompromised hosts. Several studies in AIDS patients have demonstrated that cART can produce remission of intestinal microsporidiosis.[227,228,353–355] Moreover, the declining incidence of microsporidiosis and other opportunistic infections during the cART era suggests that it also prevents symptomatic infection.

Microsporidian spores can survive and remain infective in the environment for prolonged periods. Experiments with *E. cuniculi* have demonstrated that they can survive for years in the environment with the correct humidity and temperature.[127] In the typical hospital environment, *E. cuniculi* spores can survive and remain infectious for at least 1 month. Spores can be rendered noninfectious by a 30-minute exposure to most common disinfectants, so the procedures used to clean most hospital rooms should be sufficient to limit infection. Spores are also killed by the methods commonly used for sterilization.

Although the epidemiology of the Microsporidia that infect humans has not been fully elucidated, it is likely they are foodborne or waterborne pathogens, and the usual sanitary measures that prevent contamination of food and water with animal urine and feces should decrease the chance for infection. Hand washing and general hygienic habits probably reduce the chance of contamination of the conjunctiva and cornea with microsporidian spores. It is not known whether person-to-person respiratory transmission occurs. Given the presence of microsporidian spores in respiratory secretions in cases of disseminated microsporidiosis, it may be useful to consider preventing contact of these patients with other immune-suppressed patients until the infection has been treated. Existing guidelines for the prevention of opportunistic infections that address food, water, and animal contact may be useful for preventing microsporidiosis. The presence of these organisms in genitourinary secretions raises the possibility of sexual transmission of these infections. It is reasonable to screen close contacts of patients with index cases of microsporidiosis for the presence of these organisms. Their importance and prevalence in our water supplies is an open question, but severely immunocompromised patients may wish to consider using bottled or filtered water in some settings.

Key References

The complete reference list is available online at Expert Consult.

5. Weiss LM, Becnel JJ. *Microsporidia: Pathogens of Opportunity*. Oxford: Wiley-Blackwell; 2014.
6. Capella-Gutiérrez S, Marcet-Houben M, Gabaldón T. Phylogenomics supports microsporidia as the earliest diverging clade of sequenced fungi. *BMC Biol.* 2012;10:47.
13. Desportes I, Le Charpentier Y, Galian A, et al. Occurrence of a new microsporidian: *Enterocytozoon bieneusi* n. g., n. sp., in the enterocytes of a human patient with AIDS. *J Protozool.* 1985;32:250–254.
15. Silveira H, Canning EU. *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. *J Eukaryot Microbiol.* 1995;42:158–165.
16. Cali A, Takvorian PM, Lewin S, et al. *Brachiola vesicularum,* n. g., n. sp., a new microsporidium associated with AIDS and myositis. *J Eukaryot Microbiol.* 1998;45:240–251.
18. Cali A, Kotler DP, Orenstein JM. *Septata intestinalis* n. g., n. sp., an intestinal microsporidian associated with chronic diarrhea and dissemination in AIDS patients. *J Eukaryot Microbiol.* 1993;40:101–112.
20. Field AS, Marriott DJ, Milliken ST, et al. Myositis associated with a newly described microsporidian, *Trachipleistophora hominis,* in a patient with AIDS. *J Clin Microbiol.* 1996;34:2803–2811.
22. Meissner EG, Bennett JE, Qvarnstrom Y, et al. Disseminated microsporidiosis in an immunosuppressed patient. *Emerg Infect Dis.* 2012;18:1155–1158.
23. Choudhary MM, Metcalfe MG, Arrambide K, et al. *Tubulinosema* sp. microsporidian myositis in immunosuppressed patient. *Emerg Infect Dis.* 2011;17:1727–1730.
24. Suankratay C, Thiansukhon E, Nilaratanakul V, et al. Disseminated infection caused by novel species of *Microsporidium,* Thailand. *Emerg Infect Dis.* 2012;18:302–304.
34. Cali A, Takvorian PM. Ultrastructure and development of *Pleistophora ronneafiei* n. sp., a microsporidium (Protista) in the skeletal muscle of an immune-compromised individual. *J Eukaryot Microbiol.* 2003;50:77–85.
35. Vavra J, Yachnis AT, Shadduck JA, et al. Microsporidia of the genus *Trachipleistophora*—causative agents of human microsporidiosis: description of *Trachipleistophora anthropophthera* n. sp. (Protozoa: Microsporidia). *J Eukaryot Microbiol.* 1998;45:273–283.
36. Pol S, Romana CA, Richard S, et al. Microsporidia infection in patients with the human immunodeficiency virus and unexplained cholangitis. *N Engl J Med.* 1993;328:95–99.
39. Williams BA, Hirt RP, Lucocq JM, et al. A mitochondrial remnant in the microsporidian *Trachipleistophora hominis*. *Nature.* 2002;418:865–869.

43. Xu Y, Weiss LM. The microsporidian polar tube: a highly specialised invasion organelle. *Int J Parasitol*. 2005;35:941–953.
72. Keeling PJ, Corradi N, Morrison HG, et al. The reduced genome of the parasitic microsporidian *Enterocytozoon bieneusi* lacks genes for core carbon metabolism. *Genome Biol Evol*. 2010;2:304–309.
82. Matos O, Lobo ML, Xiao L. Epidemiology of *Enterocytozoon bieneusi* infection in humans. *J Parasitol Res*. 2012;2012:981424.
91. Didier ES, Didier PJ, Friedberg DN, et al. Isolation and characterization of a new human microsporidian, *Encephalitozoon hellem* (n. sp.) from three AIDS patients with keratoconjunctivitis. *J Infect Dis*. 1991;163:617–621.
112. Corsaro D, Michel R, Walochnik J, et al. Molecular identification of *Nucleophaga terricolae* sp. nov. (Rozellomycota), and new insights on the origin of the Microsporidia. *Parasitol Res*. 2016;115:3003–3011.
117. Izquierdo F, Castro Hermida JA, Fenoy S, et al. Detection of microsporidia in drinking water, wastewater and recreational rivers. *Water Res*. 2011;45:4837–4843.
119. Decraene V, Lebbad M, Botero-Kleiven S, et al. First reported foodborne outbreak associated with microsporidia, Sweden, October 2009. *Epidemiol Infect*. 2012;140:519–527.
126. Tan J, Lee P, Lai Y, et al. Microsporidial keratoconjunctivitis after rugby tournament, Singapore. *Emerg Infect Dis*. 2013;19:1484–1486.
135. Hocevar SN, Paddock CD, Spak CW, et al. Microsporidiosis acquired through solid organ transplantation: a public health investigation. *Ann Intern Med*. 2014;160:213–220.
137. Matthis A, Weber R, Deplazes P. Zoonotic potential of the microsporidia. *Clin Microbiol Rev*. 2005;18:423–445.
158. Wanke CA, DeGirolami P, Federman M. *Enterocytozoon bieneusi* infection and diarrheal disease in patients who were not infected with human immunodeficiency virus: case report and review. *Clin Infect Dis*. 1996;23:816–818.
165. Das S, Sharma S, Sahu SK, et al. Diagnosis, clinical features and treatment outcome of microsporidial keratoconjunctivitis. *Br J Ophthalmol*. 2012;96:793–795.
180. Lanternier F, Boutboul D, Menotti J, et al. Microsporidiosis in solid organ transplant recipients: two *Enterocytozoon bieneusi* cases and review. *Transpl Infect Dis*. 2009;11:83–88.
181. Champion L, Durrbach A, Lang P, et al. Fumagillin for treatment of intestinal microsporidiosis in renal transplant recipients. *Am J Transplant*. 2010;10:1925–1930.
182. Galván AL, Sánchez AM, Valentín MA, et al. First cases of microsporidiosis in transplant recipients in Spain and review of the literature. *J Clin Microbiol*. 2011;49:1301–1306.
187. Coyle CM, Wittner M, Kotler DP, et al. Prevalence of microsporidiosis due to *Enterocytozoon bieneusi* and *Encephalitozoon (Septata) intestinalis* among patients with AIDS-related diarrhea: determination by polymerase chain reaction to the microsporidian small-subunit rRNA gene. *Clin Infect Dis*. 1996;23:1002–1006.
190. Molina JM, Oksenhendler E, Beauvais B, et al. Disseminated microsporidiosis due to *Septata intestinalis* in patients with AIDS: clinical features and response to albendazole therapy. *J Infect Dis*. 1995;171:245–249.
191. Molina JM, Tourneur M, Sarfati C, et al. Fumagillin treatment of intestinal microsporidiosis. *N Engl J Med*. 2002;346:1963–1969.
199. Van Gool T, Vetter JC, Weinmayr B, et al. High seroprevalence of *Encephalitozoon* species in immunocompetent subjects. *J Infect Dis*. 1997;175:1020–1024.
202. Didier ES, Varner PW, Didier PJ, et al. Experimental microsporidiosis in immunocompetent and immunodeficient mice and monkeys. *Folia Parasitol*. 1994;41:1–11.
217. Khan IA, Schwartzman JD, Kasper LH, et al. CD8+ CTLs are essential for protective immunity against *Encephalitozoon cuniculi* infection. *J Immunol*. 1999;162:6086–6091.
221. Moretto MM, Khan IA, Weiss LM. Gastrointestinal cell mediated immunity and the microsporidia. *PLoS Pathog*. 2012;8:e1002775.
237. Soule JB, Halverson AL, Becker RB, et al. A patient with acquired immunodeficiency syndrome and untreated *Encephalitozoon (Septata) intestinalis* microsporidiosis leading to small bowel perforation: response to albendazole. *Arch Pathol Lab Med*. 1997;121:880–887.
243. Torres G, Izquierdo F, Capó V, et al. Genital microsporidiosis in women with AIDS: a post-mortem study. *Rev Iberoam Micol*. 2013;30:47–50.
245. Pariyakanok L, Jongwutiwes S. Keratitis caused by *Trachipleistophora anthropopthera*. *J Infect*. 2005;51:325–328.
248. Pradhan S, Mascarenhas J, Srinivasan M. Microsporidial stromal keratitis masquerading as acute graft rejection. *Cornea*. 2015;34:353–354.
249. Filho MM, Ribeiro HB, Paula LJ, et al. Images in cardiovascular medicine. Endocarditis secondary to microsporidia: giant vegetation in a pacemaker user. *Circulation*. 2009;119:e386–e388. Erratum in: Circulation. 2010;121:e9.
250. Coyle CM, Weiss LM, Rhodes LV 3rd, et al. Fatal myositis due to the microsporidian *Brachiola algerae,* a mosquito pathogen. *N Engl J Med*. 2004;351:42–47.
265. Del Aguila C, Lopez-Velez R, Fenoy S, et al. Identification of *Enterocytozoon bieneusi* spores in respiratory samples from an AIDS patient with a 2-year history of intestinal microsporidiosis. *J Clin Microbiol*. 1997;35:1862–1866.
280. Ladapo TA, Nourse P, Pillay K, et al. Microsporidiosis in pediatric renal transplant patients in Cape Town, South Africa: two case reports. *Pediatr Transplant*. 2014;18:E220–E226.
281. Kicia M, Wesolowska M, Jakuszko K, et al. Concurrent infection of the urinary tract with *Encephalitozoon cuniculi* and *Enterocytozoon bieneusi* in a renal transplant recipient. *J Clin Microbiol*. 2014;52:1780–1782.
283. Nagpal A, Pritt BS, Lorenz EC, et al. Disseminated microsporidiosis in a renal transplant recipient: case report and review of the literature. *Transpl Infect Dis*. 2013;15:526–532.
285. Godron A, Accoceberry I, Couret A, et al. Intestinal microsporidiosis due to *Enterocytozoon bieneusi* in a pediatric kidney transplant recipient successfully treated with fumagillin. *Transplantation*. 2013;96:e66–e67.
286. Desoubeaux G, Maakaroun-Vermesse Z, Lier C, et al. Successful treatment with fumagillin of the first pediatric case of digestive microsporidiosis in a liver-kidney transplant. *Transpl Infect Dis*. 2013;15:E250–E259.
287. Aikawa NE, Twardowsky Ade O, Carvalho JF, et al. Intestinal microsporidiosis: a hidden risk in rheumatic disease patients undergoing anti-tumor necrosis factor therapy combined with disease-modifying anti-rheumatic drugs? *Clinics (Sao Paulo)*. 2011;66:1171–1175.
300. Watts MR, Chan RC, Cheong EY, et al. *Anncaliia algerae* microsporidial myositis. *Emerg Infect Dis*. 2014;20:185–191.
303. Loignon M, Labrecque LG, Bard C, et al. Cerebral microsporidiosis manifesting as progressive multifocal leukoencephalopathy in an HIV-infected individual—a case report. *AIDS Res Ther*. 2014;11:20.
315. Dore GJ, Marriott DJ, Hing MC, et al. Disseminated microsporidiosis due to *Septata intestinalis* in nine patients infected with the human immunodeficiency virus: response to therapy with albendazole. *Clin Infect Dis*. 1995;21:70–76.
324. Weber R, Bryan RT, Owen RL, et al. Improved light-microscopal detection of microsporidia spores in stool and duodenal aspirates. *N Engl J Med*. 1992;326:161–166.
325. Vavra J, Dahbiova R, Hollister WS, et al. Staining of microsporidian spores by optical brighteners with remarks on the use of brighteners for the diagnosis of AIDS-associated human microsporidiosis. *Folia Parasitol (Praha)*. 1993;40:267–272.
330. Didier ES, Orenstein JM, Aldras A, et al. Comparison of three staining methods for detecting microsporidia in fluids. *J Clin Microbiol*. 1995;33:3138–3145.
335. Levine DJ, Riley DJ, Jorgensen JH, et al. Key diagnostic features of granulomatous interstitial nephritis due to *Encephalitozoon cuniculi* in a lung transplant recipient. *Am J Surg Pathol*. 2013;37:447–452.
341. Sheoran AS, Feng X, Singh I, et al. Monoclonal antibodies against *Enterocytozoon bieneusi* of human origin. *Clin Diagn Lab Immunol*. 2005;12:1109–1113.
343. Visvesvara GS. In vitro cultivation of microsporidia of clinical importance. *Clin Microbiol Rev*. 2002;15:401–413.
349. Ghosh K, Weiss LM. Molecular diagnostic tests for microsporidia. *Interdiscip Perspect Infect Dis*. 2009;2009:926521.
359. Costa SF, Weiss LM. Drug treatment of microsporidiosis. *Drug Resist Update*. 2000;3:384–399.
384. Diesenhouse MC, Wilson LA, Corrent GF, et al. Treatment of microsporidial keratoconjunctivitis with topical fumagillin. *Am J Ophthalmol*. 1993;115:293–298.
386. Didier PJ, Phillips JN, Kuebler DJ, et al. Antimicrosporidial activities of fumagillin, TNP-470, ovalicin, and ovalicin derivatives in vitro and in vivo. *Antimicrob Agents Chemother*. 2006;50:2146–2155.
383. Audemard A, Le Bellec ML, Carluer L, et al. Fumagillin-induced aseptic meningoencephalitis in a kidney transplant recipient with microsporidiosis. *Transpl Infect Dis*. 2012;14:E147–E149.
394. Das S, Wallang BS, Sharma S, et al. The efficacy of corneal debridement in the treatment of microsporidial keratoconjunctivitis: a prospective randomized clinical trial. *Am J Ophthalmol*. 2014;157:1151–1155.

References

1. Sprague V. Systematics of the microsporidia. In: Bulla LA, Cheng TC, eds. *Comparative Pathobiology*. Vol. 2. New York: Plenum Press; 1977:1–510.
2. Franzen C. Microsporidia: a review of 150 years of research. *Open Parasitol J*. 2008;2:1–34.
3. Sprague W, Becnel JJ. Note on the name-author-date combination for the taxon Microsporidies Balbiani, 1882, when ranked as a phylum. *J Invertebr Pathol*. 1998;71:91–94.
4. Wittner M, Weiss LM. *The Microsporidia and Microsporidiosis*. Washington, DC: ASM Press; 1999.
5. Weiss LM, Becnel JJ. *Microsporidia: Pathogens of Opportunity*. Oxford: Wiley-Blackwell; 2014.
6. Capella-Gutiérrez S, Marcet-Houben M, Gabaldón T. Phylogenomics supports microsporidia as the earliest diverging clade of sequenced fungi. *BMC Biol*. 2012;10:47.
7. Lee SC, Corradi N, Byrnes EJ 3rd, et al. Microsporidia evolved from ancestral sexual fungi. *Curr Biol*. 2008;18:1675–1679.
8. Black SS, Steinohrt LA, Bertucci DC, et al. *Encephalitozoon hellem* in budgerigars *(Melopsittacus undulatus)*. *Vet Pathol*. 1997;34:189–198.
9. American Mosquito Control Association. Biological control of mosquitoes. AMCA Bull No. 6. Fresno, CA: American Mosquito Control Association; 1985:218.
10. Weber R, Bryan RT, Schwartz DA, et al. Human microsporidial infections. *Clin Microbiol Rev*. 1994;7:426–461.
11. Levaditi C, Nicolau S, Schoen R. L'agent etiologique de l'enchalite epizootique du lapin *(Encephalitozoon cuniculi)*. *C R Soc Biol*. 1923;89:984–986.
12. Matsubayashi H, Koide T, Mikata T, et al. A case of *Encephalitozoon*-like body infection in man. *Arch Pathol Lab Med*. 1959;67:181–185.
13. Desportes I, Le Charpentier Y, Galian A, et al. Occurrence of a new microsporidian: *Enterocytozoon bieneusi* n. g., n. sp., in the enterocytes of a human patient with AIDS. *J Protozool*. 1985;32:250–254.
14. Sprague V, Becnel JJ, Hazard EI. Taxonomy of phylum Microspora. *Crit Rev Microbiol*. 1992;18:285–395.
15. Silveira H, Canning EU. *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. *J Eukaryot Microbiol*. 1995;42:158–165.
16. Cali A, Takvorian PM, Lewin S, et al. *Brachiola vesicularum,* n. g., n. sp., a new microsporidium associated with AIDS and myositis. *J Eukaryot Microbiol*. 1998;45:240–251.
17. Franzen C, Nassonova ES, Scholmerich J, et al. Transfer of the genus *Brachiola* (microsporidia) to the genus *Anncaliia* based on ultrastructural and molecular data. *J Eukaryot Microbiol*. 2006;53:26–35.
18. Cali A, Kotler DP, Orenstein JM. *Septata intestinalis* n. g., n. sp., an intestinal microsporidian associated with chronic diarrhea and dissemination in AIDS patients. *J Eukaryot Microbiol*. 1993;40:101–112.
19. Hartskeerl RA, Van Gool T, Schuitema AR, et al. Genetic and immunological characterization of the microsporidian *Septata intestinalis* Cali, Kotler and Orenstein, 1993: reclassification to *Encephalitozoon intestinalis*. *Parasitology*. 1995;110:277–285.
20. Field AS, Marriott DJ, Milliken ST, et al. Myositis associated with a newly described microsporidian, *Trachipleistophora hominis,* in a patient with AIDS. *J Clin Microbiol*. 1996;34:2803–2811.
21. Yachnis AT, Berg J, Martinez-Salazar A, et al. Disseminated microsporidiosis especially infecting the brain, heart, and kidneys: report of a newly recognized pansporoblastic species in two symptomatic AIDS patients. *Am J Clin Pathol*. 1996;106:535–543.
22. Meissner EG, Bennett JE, Qvarnstrom Y, et al. Disseminated microsporidiosis in an immunosuppressed patient. *Emerg Infect Dis*. 2012;18:1155–1158.
23. Choudhary MM, Metcalfe MG, Arrambide K, et al. *Tubulinosema* sp. microsporidian myositis in immunosuppressed patient. *Emerg Infect Dis*. 2011;17:1727–1730.
24. Suankratay C, Thiansukhon E, Nilaratanakul V, et al. Disseminated infection caused by novel species of *Microsporidium,* Thailand. *Emerg Infect Dis*. 2012;18:302–304.
25. Rastrelli P, Didier E, Yee R. Microsporidial keratitis. *Ophthalmol Clin North Am*. 1994;7:614–635.
26. Orenstein JM, Gaetz HP, Yachnis AT, et al. Disseminated microsporidiosis in AIDS: are any organs spared? *AIDS*. 1997;11:385–386.
27. Sheth SG, Bates C, Federman M, et al. Fulminant hepatic failure caused by microsporidial infection in a patient with AIDS. *AIDS*. 1997;11:553–554.
28. Weber R, Deplazes P, Flepp M, et al. Cerebral microsporidiosis due to *Encephalitozoon cuniculi* in a patient with human immunodeficiency virus infection. *N Engl J Med*. 1997;336:474–478.
29. Sheikh RA, Prindiville TP, Yenamandra S, et al. Microsporidial AIDS cholangiopathy due to *Encephalitozoon intestinalis:* case report and review. *Am J Gastroenterol*. 2000;95:2364–2371.
30. Visvesvara GS, da Silva AJ, Croppo GP, et al. In vitro culture and serologic and molecular identification of *Septata intestinalis* isolated from urine of a patient with AIDS. *J Clin Microbiol*. 1995;33:930–936.
31. Shadduck JA, Meccoli RA, Davis R, et al. Isolation of a microsporidian from a human patient. *J Infect Dis*. 1990;162:773–776.
32. Cali A, Takvorian PM, Lewin S, et al. Identification of a new *Nosema*-like microsporidian associated with myositis in an AIDS patient. *J Eukaryot Microbiol*. 1996;43:108S.
33. Chupp GL, Alroy J, Adelman LS, et al. Myositis due to *Pleistophora* (Microsporidia) in a patient with AIDS. *Clin Infect Dis*. 1993;16:15–21.
34. Cali A, Takvorian PM. Ultrastructure and development of *Pleistophora ronneafiei* n. sp., a microsporidium (Protista) in the skeletal muscle of an immune-compromised individual. *J Eukaryot Microbiol*. 2003;50:77–85.
35. Vavra J, Yachnis AT, Shadduck JA, et al. Microsporidia of the genus *Trachipleistophora*—causative agents of human microsporidiosis: description of *Trachipleistophora anthropophthera* n. sp. (Protozoa: Microsporidia). *J Eukaryot Microbiol*. 1998;45:273–283.
36. Pol S, Romana CA, Richard S, et al. Microsporidia infection in patients with the human immunodeficiency virus and unexplained cholangitis. *N Engl J Med*. 1993;328:95–99.
37. Franzen C, Muller A. Microsporidiosis: human diseases and diagnosis. *Microbes Infect*. 2001;3:389–400.
38. Desportes-Livage I. Biology of microsporidia. *Contrib Microbiol*. 2000;6:140–165.
39. Williams BA, Hirt RP, Lucocq JM, et al. A mitochondrial remnant in the microsporidian *Trachipleistophora hominis*. *Nature*. 2002;418:865–869.
40. Weber R, Deplazes P, Schwartz D. Diagnosis and clinical aspects of human microsporidiosis. *Contrib Microbiol*. 2000;6:166–192.
41. Vavra J. Structure of the microsporidia. In: Bulla LA Jr, Cheng TC, eds. *Comparative Pathobiology*. Vol. 1. New York: Plenum Press; 1976:1–85.
42. Southern TR, Jolly CE, Lester ME, et al. EnP1, a microsporidian spore wall protein that enables spores to adhere to and infect host cells in vitro. *Eukaryot Cell*. 2007;6:1354–1362.
43. Xu Y, Weiss LM. The microsporidian polar tube: a highly specialised invasion organelle. *Int J Parasitol*. 2005;35:941–953.
44. Ghosh K, Nieves E, Keeling P, et al. Branching network of proteinaceous filaments within the parasitophorous vacuole of *Encephalitozoon cuniculi* and *Encephalitozoon hellem*. *Infect Immun*. 2011;79:1374–1385.
45. Li Z, Pan G, Li T, et al. SWP5, a spore wall protein, interacts with polar tube proteins in the parasitic microsporidian *Nosema bombycis*. *Eukaryot Cell*. 2012;11:229–237.
46. Xu Y, Takvorian PM, Cali A, et al. Glycosylation of the major polar tube protein of *Encephalitozoon hellem*, a microsporidian parasite that infects humans. *Infect Immun*. 2004;72:6341–6350.
47. Xu Y, Takvorian P, Cali A, et al. Identification of a new spore wall protein from *Encephalitozoon cuniculi*. *Infect Immun*. 2006;74:239–247.
48. Bouzahzah B, Weiss LM. Glycosylation of the major polar tube protein of *Encephalitozoon cuniculi*. *Parasitol Res*. 2010;107:761–764.
49. Bouzahzah B, Nagajyothi F, Ghosh K, et al. Interactions of *Encephalitozoon cuniculi* polar tube proteins. *Infect Immun*. 2010;78:2745–2753.
50. Foucault C, Drancourt M. Actin mediates *Encephalitozoon intestinalis* entry into the human enterocyte-like cell line, Caco-2. *Microb Pathog*. 2000;28:51–58.
51. Lom J. On the structure of the extruded microsporidian polar filament. *Z Parasitenkd*. 1972;38:200–213.
52. Weidner E. Ultrastructural study of microsporidian invasion into cells. *N Parasitenkd*. 1972;40:227–242.
53. Undeen AH, Frixione E. The role of osmotic pressure in the germination of *Nosema algerae* spores. *J Protozool*. 1990;37:561–567.
54. Lom J, Vavra J. The mode of sporoplasm extrusion in microsporidian spores. *Acta Protozool*. 1963;1:81–89.
55. Keohane E, Weiss LM. The structure, function, and composition of the microsporidian polar tube. In: Wittner M, Weiss LM, eds. *The Microsporidia and Microsporidiosis*. Washington, DC: ASM Press; 1999:196–224.
56. Undeen AH, Solter LF. Sugar acquisition during the development of microsporidian (Microspora: Sosematidae) spores. *J Invertebr Pathol*. 1997;70:106–112.
57. Undeen AH, Vander Meer RK. Microsporidian intrasporal sugars and their role in germination. *J Invertebr Pathol*. 1999;73:294–302.
58. Curgy JJ, Vavra J, Vivares C. Presence of ribosomal RNAs with prokaryotic properties in Microsporidia, eukaryotic organisms. *Biol Cell*. 1980;38:49–52.
59. Vossbrinck CR, Woese CR. Eukaryotic ribosomes that lack a 5.8S RNA. *Nature*. 1986;320:287–288.
60. Vossbrinck CR, Maddox JV, Friedman S, et al. Ribosomal RNA sequence suggests microsporidia are extremely ancient eukaryotes. *Nature*. 1987;326:411–414.
61. Weiss LM, Vossbrinck CR. Microsporidiosis: molecular and diagnostic aspects. *Adv Parasitol*. 1998;40:351–395.
62. Vivares CP, Metenier G. Towards the minimal eukaryotic parasitic genome. *Curr Opin Microbiol*. 2000;3:463–467.
63. Brugere JF, Cornillot E, Metenier G, et al. *Encephalitozoon cuniculi* (Microspora) genome: physical map and evidence for telomere-associated rDNA units on all chromosomes. *Nucleic Acids Res*. 2000;28:2026–2033.
64. Katiyar SK, Visvesvara GS, Edlind TD. Comparisons of ribosomal RNA sequences from amitochondrial protozoa: implications for processing, mRNA binding and paromomycin susceptibility. *Gene*. 1995;152:27–33.
65. Weiss LM, Vossbrinck CR. Molecular biology, molecular phylogeny, and molecular diagnostic approaches to the microsporidia. In: Wittner M, Weiss LM, eds. *The Microsporidia and Microsporidiosis*. Washington, DC: ASM Press; 1999:129–171.
66. Corradi N, Akiyoshi DE, Morrison HG, et al. Patterns of genome evolution among the microsporidian parasites *Encephalitozoon cuniculi*, *Antonospora locustae* and *Enterocytozoon bieneusi*. *PLoS ONE*. 2007;2:e1277.
67. Aurrecoechea C, Barreto A, Brestelli J, et al. AmoebaDB and MicrosporidiaDB: functional genomic resources for Amoebozoa and Microsporidia species. *Nucleic Acids Res*. 2011;39:D612–D619.
68. Katinka MD, Duprat S, Cornillot E, et al. Genome sequence and gene compaction of the eukaryote parasite *Encephalitozoon cuniculi*. *Nature*. 2001;414:450–453.
69. Pombert JF, Xu J, Smith DR, et al. Complete genome sequences from three genetically distinct strains reveal a high intra-species genetic diversity in the microsporidian *Encephalitozoon cuniculi*. *Eukaryot Cell*. 2013;12:503–511.
70. Pombert JF, Selman M, Burki F, et al. Gain and loss of multiple functionally related, horizontally transferred genes in the reduced genomes of two microsporidian parasites. *Proc Natl Acad Sci USA*. 2012;109:12638–12643.
71. Corradi N, Pombert JF, Farinelli L, et al. The complete sequence of the smallest known nuclear genome from the microsporidian *Encephalitozoon intestinalis*. *Nat Commun*. 2010;1:77.
72. Keeling PJ, Corradi N, Morrison HG, et al. The reduced genome of the parasitic microsporidian *Enterocytozoon bieneusi* lacks genes for core carbon metabolism. *Genome Biol Evol*. 2010;2:304–309.
73. Akiyoshi DE, Morrison HG, Lei S, et al. Genomic survey of the non-cultivatable opportunistic human pathogen, *Enterocytozoon bieneusi*. *PLoS Pathog*. 2009;5:e1000261.
74. Brugere JF, Cornillot E, Metenier G, et al. Occurrence of subtelomeric rearrangements in the genome of the microsporidian parasite *Encephalitozoon cuniculi,* as revealed by a new fingerprinting procedure based on two-dimensional pulsed field gel electrophoresis. *Electrophoresis*. 2000;21:2576–2581.
75. Selman M, Sak B, Kvác M, et al. Extremely reduced levels of heterozygosity in the vertebrate pathogen *Encephalitozoon cuniculi*. *Eukaryot Cell*. 2013;2:496–502.
76. Cuomo CA, Desjardins CA, Bakowski MA, et al. Microsporidian genome analysis reveals evolutionary strategies for obligate intracellular growth. *Genome Res*. 2012;22:2478–2488.
77. Vossbrinck CF, Maddox JV, Friedman S, et al. Ribosomal RNA sequence suggests microsporidia are extremely ancient eukaryotes. *Nature*. 1987;326:411–414.
78. Baker MD, Vossbrinck CR, Maddox JV, et al. Phylogenetic relationships among *Vairimorpha* and *Nosema* species (Microspora) based on ribosomal RNA sequence data. *J Invertebr Pathol*. 1994;64:100–106.
79. Franzen C, Muller A. Molecular techniques for detection, species differentiation, and phylogenetic analysis of microsporidia. *Clin Microbiol Rev*. 1999;12:243–285.
80. Didier ES, Vossbrinck CR, Baker MD, et al. Identification and characterization of three *Encephalitozoon cuniculi* strains. *Parasitology*. 1995;111:411–421.
81. Rinder H, Thomschke A, Dengjel B, et al. Close genotypic relationship between *Enterocytozoon bieneusi* from humans and pigs and first detection in cattle. *J Parasitol*. 2000;86:185–188.

82. Matos O, Lobo ML, Xiao L. Epidemiology of *Enterocytozoon bieneusi* infection in humans. *J Parasitol Res*. 2012;2012:981424.
83. Li W, Cama V, Feng Y, et al. Population genetic analysis of *Enterocytozoon bieneusi* in humans. *Int J Parasitol*. 2012;42:287–293.
84. Santín M, Fayer R. *Enterocytozoon bieneusi* genotype nomenclature based on the internal transcribed spacer sequence: a consensus. *J Eukaryot Microbiol*. 2009;56:34–38.
85. Tuzet O, Maurand J, Fize JA, et al. Proposition d'un nouveau cadre systematique pour les genres de Microsporidies. *C R Acad Sci (Paris)*. 1971;272:1268–1271.
86. Larsson JIR. Identification of microsporidian genera: a guide with comments on the taxonomy. *Arch Protistenkd*. 1988;136:1–37.
87. Issi IV. Microsporidia as a phylum of parasitic protozoa [in Russian]. *Protozoology*. 1986;10:6–135.
88. Weiser J. A proposal of the basis for microsporidian taxonomy. *Proc Int Congr Protozool*. 1977;5:267.
89. Baker MD. *Phylogenetic Relationships of Five Microsporidian Genera Based on Ribosomal RNA Sequence Data*. Urbana-Champaign: University of Illinois; 1987.
90. Vossbrinck CR, Debrunner-Vossbrinck BA. Molecular phylogeny of the Microsporidia: ecological, ultrastructural and taxonomic considerations. *Folia Parasitol (Praha)*. 2005;52:131–142, discussion 130.
91. Didier ES, Didier PJ, Friedberg DN, et al. Isolation and characterization of a new human microsporidian, *Encephalitozoon hellem* (n. sp.) from three AIDS patients with keratoconjunctivitis. *J Infect Dis*. 1991;163:617–621.
92. Baker MD, Vossbrinck CR, Didier ES, et al. Small subunit ribosomal DNA phylogeny of various microsporidia with emphasis on AIDS related forms. *J Eukaryot Microbiol*. 1995;42:564–570.
93. Keeling PJ, McFadden GI. Origins of microsporidia. *Trends Microbiol*. 1998;6:19–23.
94. Weiss LM, Edlind TD, Vossbrinck CR, et al. Microsporidian molecular phylogeny: the fungal connection. *J Eukaryot Microbiol*. 1999;46:17S–18S.
95. Hirt RP, Logsdon JM Jr, Healy B, et al. Microsporidia are related to fungi: evidence from the largest subunit of RNA polymerase II and other proteins. *Proc Natl Acad Sci USA*. 1999;96:580–585.
96. Edlind T, Katiyar S, Visvesvara G, et al. Evolutionary origins of microsporidia and basis for benzimidazole sensitivity: an update. *J Eukaryot Microbiol*. 1996;43:109S.
97. Keeling PJ, Doolittle WF. Alpha-tubulin from early-diverging eukaryotic lineages and the evolution of the tubulin family. *Mol Biol Evol*. 1996;13:1297–1305.
98. Arisue N, Sanchez LB, Weiss LM, et al. Mitochondrial-type hsp70 genes of the amitochondriate protists, *Giardia intestinalis, Entamoeba histolytica* and two microsporidians. *Parasitol Int*. 2002;51:9–16.
99. Germot A, Philippe H, Le GH. Evidence for loss of mitochondria in microsporidia from a mitochondrial-type hsp70 in *Nosema locusta*e. *Mol Biochem Parasitol*. 1997;87:159–168.
100. Hirt RP, Healy B, Vossbrinck CR, et al. A mitochondrial Hsp70 orthologue in *Vairimorpha necatrix:* molecular evidence that microsporidia once contained mitochondria. *Curr Biol*. 1997;7:995–998.
101. Peyretaillade E, Broussolle V, Peyret P, et al. Microsporidia, amitochondrial protists, possess a 70-kDa heat shock protein gene of mitochondrial evolutionary origin. *Mol Biol Evol*. 1998;15:683–689.
102. Vivares C, Biderre C, Duffieux F, et al. Chromosomal localization of five genes in *Encephalitozoon cuniculi* (Microsporidia). *J Eukaryot Microbiol*. 1996;43:97S.
103. Edlind T. Phylogenetics of protozoan tubulin with reference to the amitochondriate eukaryotes. In: Coombs GH, Vickerman K, Sleigh MA, et al, eds. *Evolutionary Relationships Among Protozoa*. London: Chapman & Hall; 1998:91–108.
104. Kamaishi T, Hashimoto T, Nakamura Y, et al. Protein phylogeny of translation elongation factor EF-1 alpha suggests microsporidians are extremely ancient eukaryotes. *J Mol Evol*. 1996;42:257–263.
105. Desportes I. Ultratrastructure de *Stempellia mutabilis* Leger et Hess, microsporidie parasite de l'ephemere *Ephemera vulgatta*. *L Protistologica*. 1976;12:121–150.
106. Flegel TW, Pasharawipas T. A proposal for typical eukaryotic meiosis in microsporidians. *Can J Microbiol*. 1995;41:1–11.
107. Fast NM, Logsdon JM Jr, Doolittle WF. Phylogenetic analysis of the TATA box binding protein (TBP) gene from *Nosema locustae:* evidence for a microsporidia-fungi relationship and spliceosomal intron loss. *Mol Biol Evol*. 1999;16:1415–1419.
108. Keeling PJ. Congruent evidence from alpha-tubulin and beta-tubulin gene phylogenies for a zygomycete origin of microsporidia. *Fungal Genet Biol*. 2003;38:298–309.
109. Lee SC, Weiss LM, Heitman J. Generation of genetic diversity in microsporidia via sexual reproduction and horizontal gene transfer. *Commun Integr Biol*. 2009;2:414–417.
110. Lee SC, Corradi N, Doan S, et al. Evolution of the sex-related locus and genomic features shared in microsporidia and fungi. *PLoS ONE*. 2010;5:e10539.
111. Jones MD, Forn I, Gadelha C, et al. Discovery of novel intermediate forms redefines the fungal tree of life. *Nature*. 2011;474:200–203.
112. Corsaro D, Michel R, Walochnik J, et al. Molecular identification of *Nucleophaga terricolae* sp. nov. (Rozellomycota), and new insights on the origin of the Microsporidia. *Parasitol Res*. 2016;115:3003–3011.
113. Avery SW, Undeen AH. The isolation of microsporidia and other pathogens from concentrated ditch water. *J Am Mosq Control Assoc*. 1987;3:54–58.
114. Cotte L, Rabodonirina M, Chapuis F, et al. Waterborne outbreak of intestinal microsporidiosis in persons with and without human immunodeficiency virus infection. *J Infect Dis*. 1999;180:2003–2008.
115. Sparfel JM, Sarfati C, Liguory O, et al. Detection of microsporidia and identification of *Enterocytozoon bieneusi* in surface water by filtration followed by specific PCR. *J Eukaryot Microbiol*. 1997;44:78S.
116. Dowd SE, Gerba CP, Pepper IL. Confirmation of the human-pathogenic microsporidia *Enterocytozoon bieneusi*, *Encephalitozoon intestinalis*, and *Vittaforma corneae* in water. *Appl Environ Microbiol*. 1998;64:3332–3335.
117. Izquierdo F, Castro Hermida JA, Fenoy S, et al. Detection of microsporidia in drinking water, wastewater and recreational rivers. *Water Res*. 2011;45:4837–4843.
118. Galván AL, Magnet A, Izquierdo F, et al. Molecular characterization of human-pathogenic microsporidia and *Cyclospora cayetanensis* isolated from various water sources in Spain: a year-long longitudinal study. *Appl Environ Microbiol*. 2013;79:449–459.
119. Decraene V, Lebbad M, Botero-Kleiven S, et al. First reported foodborne outbreak associated with microsporidia, Sweden, October 2009. *Epidemiol Infect*. 2012;140:519–527.
120. Enriquez FJ, Taren D, Cruz-Lopez A, et al. Prevalence of intestinal encephalitozoonosis in Mexico. *Clin Infect Dis*. 1998;26:1227–1229.
121. Hutin YJ, Sombardier MN, Liguory O, et al. Risk factors for intestinal microsporidiosis in patients with human immunodeficiency virus infection: a case-control study. *J Infect Dis*. 1998;178:904–907.
122. Conteas CN, Berlin OG, Lariviere MJ, et al. Examination of the prevalence and seasonal variation of intestinal microsporidiosis in the stools of persons with chronic diarrhea and human immunodeficiency virus infection. *Am J Trop Med Hyg*. 1998;58:559–561.
123. Wuhib T, Silva TMJ, Newman RD, et al. Cryptosporidial and microsporidial infections in human immunodeficiency virus-infected patients in northeastern Brazil. *J Infect Dis*. 1994;170:494–497.
124. Fan NW, Wu CC, Chen TL, et al. Microsporidial keratitis in patients with hot springs exposure. *J Clin Microbiol*. 2012;50:414–418.
125. Chan KS, Koh TH. Microsporidian eye infection from outdoor recreational activities. *J Clin Microbiol*. 2015;5:753.
126. Tan J, Lee P, Lai Y, et al. Microsporidial keratoconjunctivitis after rugby tournament, Singapore. *Emerg Infect Dis*. 2013;19:1484–1486.
127. Waller T. Sensitivity of *Encephalitozoon cuniculi* to various temperatures, disinfectants and drugs. *Lab Anim*. 1979;13:227–230.
128. Li X, Fayer R. Infectivity of microsporidian spores exposed to temperature extremes and chemical disinfectants. *J Eukayot Microbiol*. 2006;53(suppl 1):S77–S79.
129. Schwartz DA, Visvesvara GS, Diesenhouse MC, et al. Pathologic features and immunofluorescent antibody demonstration of ocular microsporidiosis *(Encephalitozoon hellem)* in seven patients with acquired immunodeficiency syndrome. *Am J Ophthalmol*. 1993;115:285–292.
130. Fuentealba IC, Mahoney NT, Shadduck JA, et al. Hepatic lesions in rabbits infected with *Encephalitozoon cuniculi* administered per rectum. *Vet Pathol*. 1992;29:536–540.
131. Schwartz DA, Visvesvara G, Weber R, et al. Male genital tract microsporidiosis and AIDS: prostatic abscess due to *Encephalitozoon hellem*. *J Eukaryot Microbiol*. 1994;41:61S.
132. Schwartz DA, Bryan RT, Hewanlowe KO, et al Disseminated microsporidiosis and AIDS; pathologic evidence for respiratory transmission of *Encephalitozoon* infection. In: Proceedings of the International Conference on AIDS, 1992, vol. 8.
133. Bryan RT, Schwartz DA. Epidemiology of microsporidiosis. In: Wittner M, Weiss LM, eds. *The Microsporidia and Microsporidiosis*. Washington, DC: American Society of Microbiology Press; 1999:502–516.
134. Hunt RD, King NW, Foster HL. Encephalitozoonosis: evidence for vertical transmission. *J Infect Dis*. 1972;126:212–214.
135. Hocevar SN, Paddock CD, Spak CW, et al. Microsporidiosis acquired through solid organ transplantation: a public health investigation. *Ann Intern Med*. 2014;160:213–220.
136. Smith RM, Muehlenbachs A, Schaenmann J, et al. Three cases of neurologic syndrome caused by donor-derived microsporidiosis. *Emerg Infect Dis*. 2017;23:387–395.
137. Matthis A, Weber R, Deplazes P. Zoonotic potential of the microsporidia. *Clin Microbiol Rev*. 2005;18:423–445.
138. Deplazes P, Mathis A, Weber R. Epidemiology and zoonotic aspects of microsporidia of mammals and birds. *Contrib Microbiol*. 2000;6:236–260.
139. Yee RW, Tio FO, Martinez JA, et al. Resolution of microsporidial epithelial keratopathy in a patient with AIDS. *Ophthalmology*. 1991;98:196–201.
140. Deplazes P, Mathis A, Muller C, et al. Molecular epidemiology of *Encephalitozoon cuniculi* and first detection of *Enterocytozoon bieneusi* in faecal samples of pigs. *J Eukaryot Microbiol*. 1996;43:93S.
141. Del Aguila C, Izquierdo F, Navajas R, et al. *Enterocytozoon bieneusi* in animals: rabbits and dogs as new hosts. *J Eukaryot Microbiol*. 1999;46:8S–9S.
142. Reetz J, Rinder H, Thomschke A, et al. First detection of the microsporidium *Enterocytozoon bieneusi* in non-mammalian hosts (chickens). *Int J Parasitol*. 2002;32:785–787.
143. Graczyk TK, Sunderland D, Rule AM, et al. Urban feral pigeons *(Columba livia)* as a source for air- and waterborne contamination with *Enterocytozoon bieneusi* spores. *Appl Environ Microbiol*. 2007;73:4357–4358.
144. Mansfield KG, Carville A, Shvetz D, et al. Identification of an *Enterocytozoon bieneusi*-like microsporidian parasite in simian-immunodeficiency-virus-inoculated macaques with hepatobiliary disease. *Am J Pathol*. 1997;150:1395–1405.
145. Deplazes P, Mathis A, van Saanen M, et al. Dual microsporidial infection due to *Vittaforma corneae* and *Encephalitozoon hellem* in a patient with AIDS. *Clin Infect Dis*. 1998;27:1521–1524.
146. Weber R, Bryan RT. Microsporidial infections in immunodeficient and immunocompetent patients. *Clin Infect Dis*. 1994;19:517–521.
147. Cama VA, Pearson J, Cabrera L, et al. Transmission of *Enterocytozoon bieneusi* between a child and guinea pigs. *J Clin Microbiol*. 2007;45:2708–2710.
148. Drobniewski F, Kelly P, Carew A, et al. Human microsporidiosis in African AIDS patients with chronic diarrhea. *J Infect Dis*. 1995;171:515–516.
149. Aoun K, Bouratbine A, Datry A, et al. Presence of intestinal microsporidia in Tunisia: a case report. *Bull Soc Pathol Exot*. 1997;90:176.
150. Hautvast JL, Tolboom JJ, Derks TJ, et al. Asymptomatic intestinal microsporidiosis in a human immunodeficiency virus-seronegative, immunocompetent Zambian child. *Pediatr Infect Dis J*. 1997;16:415–416.
151. Van Gool T, Luderhoff E, Nathoo KJ, et al. High prevalence of *Enterocytozoon bieneusi* infections among HIV-positive individuals with persistent diarrhoea in Harare, Zimbabwe. *Trans R Soc Trop Med Hyg*. 1995;89:478–480.
152. Morakote N, Siriprasert P, Piangjai S, et al. *Microsporidium* and *Cyclospora* in human stools in Chiang Mai, Thailand. *Southeast Asian J Trop Med Public Health*. 1995;26:799–800.
153. Brazil P, Sodre FC, Cuzzi-Maya T, et al. Intestinal microsporidiosis in HIV-positive patients with chronic unexplained diarrhea in Rio de Janeiro, Brazil: diagnosis, clinical presentation and follow-up. *Rev Inst Med Trop Sao Paulo*. 1996;38:97–102.
154. Weitz JC, Botehlo R, Bryan R. Microsporidiosis in patients with chronic diarrhea and AIDS, in HIV asymptomatic patients and in patients with acute diarrhea. *Rev Med Chil*. 1995;123:849–856.
155. Sak B, Kvac M, Sucerova Z, et al. Latent microsporidia infection in immunocompetent individuals—a longitudinal study. *PLoS Negl Trop Dis*. 2011;5:e1162.
156. Cegielski JP, Ortega YR, McKee S, et al. *Cryptosporidium, Enterocytozoon,* and *Cyclospora* infections in pediatric and adult patients with diarrhea in Tanzania. *Clin Infect Dis*. 1999;28:314–321.
157. Maiga I, Doumbo O, Dembele M, et al. Human intestinal microsporidiosis in Bamako (Mali): the presence of *Enterocytozoon bieneusi* in HIV seropositive patients. *Sante*. 1997;7:257–262.
158. Wanke CA, DeGirolami P, Federman M. *Enterocytozoon bieneusi* infection and diarrheal disease in patients who

were not infected with human immunodeficiency virus: case report and review. *Clin Infect Dis*. 1996;23:816–818.
159. Gainzarain JC, Canut A, Lozano M, et al. Detection of *Enterocytozoon bieneusi* in two human immunodeficiency virus-negative patients with chronic diarrhea by polymerase chain reaction in duodenal biopsy specimens and review. *Clin Infect Dis*. 1998;27:394–398.
160. Albrecht H, Sobottka I. *Enterocytozoon bieneusi* infection in patients who are not infected with human immunodeficiency virus. *Clin Infect Dis*. 1997;25:344.
161. Sandfort J, Hannemann A, Gelderblom H, et al. *Enterocytozoon bieneusi* infection in an immunocompetent patient who had acute diarrhea and who was not infected with the human immunodeficiency virus. *Clin Infect Dis*. 1994;19:514–516.
162. Sobottka I, Albrecht H, Schottelius J, et al. Self-limited traveller's diarrhea due to a dual infection with *Enterocytozoon bieneusi* and *Cryptosporidium parvum* in an immunocompetent HIV-negative child. *Eur J Clin Microbiol Infect Dis*. 1995;14:919–920.
163. Wichro E, Hoetzl D, Krause R, et al. Microsporidiosis in travel-associated chronic diarrhea in immune-competent patients. *Am J Trop Med Hyg*. 2005;73:285–287.
164. Raynaud L, Delbac F, Broussolle V, et al. Identification of *Encephalitozoon intestinalis* in travelers with chronic diarrhea by specific PCR amplification. *J Clin Microbiol*. 1998;36:37–40.
165. Das S, Sharma S, Sahu SK, et al. Diagnosis, clinical features and treatment outcome of microsporidial keratoconjunctivitis. *Br J Ophthalmol*. 2012;96:793–795.
166. Fan NW, Wu CC, Chen TL, et al. Microsporidial keratitis in patients with hot springs exposure. *J Clin Microbiol*. 2012;50:414–418.
167. Tham AC, Sanjay S. Clinical spectrum of microsporidial keratoconjunctivitis. *Clin Experiment Ophthalmol*. 2012;40:512–518.
168. Loh RS, Chan CM, Ti SE, et al. Emerging prevalence of microsporidial keratitis in Singapore: epidemiology, clinical features, and management. *Ophthalmology*. 2009;116:2348–2353.
169. Sharma S, Das S, Joseph J, et al. Microsporidial keratitis: need for increased awareness. *Surv Ophthalmol*. 2011;56:1–22.
170. Rabodonirina M, Bertocchi M, Desportes-Livage I, et al. *Enterocytozoon bieneusi* as a cause of chronic diarrhea in a heart-lung transplant recipient who was seronegative for human immunodeficiency virus. *Clin Infect Dis*. 1996;23:114–117.
171. Sax PE, Rich JD, Pieciak WS, et al. Intestinal microsporidiosis occurring in a liver transplant recipient. *Transplantation*. 1995;60:617–618.
172. Gumbo T, Hobbs RE, Carlyn C, et al. Microsporidia infection in transplant patients. *Transplantation*. 1999;67:482–484.
173. Kelkar R, Sastry PS, Kulkarni SS, et al. Pulmonary microsporidial infection in a patient with CML undergoing allogeneic marrow transplant. *Bone Marrow Transplant*. 1997;19:179–182.
174. Mahmood MN, Keohane ME, Burd EM. Pathologic quiz case: a 45-year-old renal transplant recipient with persistent fever. *Arch Pathol Lab Med*. 2003;127:224–226.
175. Metge S, Van Nhieu JT, Dahmane D, et al. A case of *Enterocytozoon bieneusi* infection in an HIV-negative renal transplant recipient. *Eur J Clin Microbiol Infect Dis*. 2000;19:221–223.
176. Mohindra AR, Lee MW, Visvesvara G, et al. Disseminated microsporidiosis in a renal transplant recipient. *Transpl Infect Dis*. 2002;4:102–107.
177. Sing A, Tybus K, Heesemann J, et al. Molecular diagnosis of an *Enterocytozoon bieneusi* human genotype C infection in a moderately immunosuppressed human immunodeficiency virus seronegative liver-transplant recipient with severe chronic diarrhea. *J Clin Microbiol*. 2001;39:2371–2372.
178. Field AS, Paik JY, Stark D, et al. Myositis due to the microsporidian *Anncaliia (Brachiola) algerae* in a lung transplant recipient. *Transpl Infect Dis*. 2012;14:169–176.
179. George B, Coates T, McDonald S, et al. Disseminated microsporidiosis with *Encephalitozoon* species in a renal transplant recipient. *Nephrology (Carlton)*. 2012;17(suppl 1):5–8.
180. Lanternier F, Boutboul D, Menotti J, et al. Microsporidiosis in solid organ transplant recipients: two *Enterocytozoon bieneusi* cases and review. *Transpl Infect Dis*. 2009;11:83–88.
181. Champion L, Durrbach A, Lang P, et al. Fumagillin for treatment of intestinal microsporidiosis in renal transplant recipients. *Am J Transplant*. 2010;10:1925–1930.
182. Galván AL, Sánchez AM, Valentín MA, et al. First cases of microsporidiosis in transplant recipients in Spain and review of the literature. *J Clin Microbiol*. 2011;49:1301–1306.
183. Silverstein BE, Cunningham ETJ, Margolis TP, et al. Microsporidial keratoconjunctivitis in a patient without human immunodeficiency virus infection. *Am J Ophthalmol*. 1997;124:395–396.
184. Weiss LM. And now microsporidiosis. *Ann Intern Med*. 1995;123:954–956.
185. Van Gool T, Dankert J. Human microsporidiosis: clinical, diagnostic and therapeutic aspects of an increasing infection. *Clin Microbiol Infect*. 1995;1:75–85.
186. Bryan RT, Cali A, Owen RL, et al. Microsporidia: opportunistic pathogens in patients with AIDS. In: Sun T, ed. *Progress in Clinical Parasitology*. Vol. 2. New York: Field & Wood; 1991:1–26.
187. Coyle CM, Wittner M, Kotler DP, et al. Prevalence of microsporidiosis due to *Enterocytozoon bieneusi* and *Encephalitozoon (Septata) intestinalis* among patients with AIDS-related diarrhea: determination by polymerase chain reaction to the microsporidian small-subunit rRNA gene. *Clin Infect Dis*. 1996;23:1002–1006.
188. Voglino MC, Donelli G, Rossi P, et al. Intestinal microsporidiosis in Italian individuals with AIDS. *Ital J Gastroenterol*. 1996;28:381–386.
189. Kyaw T, Curry A, Edwards-Jones V, et al. The prevalence of *Enterocytozoon bieneusi* in acquired immunodeficiency syndrome (AIDS) patients from the northwest of England: 1992-1995. *Br J Biomed Sci*. 1997;54:186–191.
190. Molina JM, Oksenhendler E, Beauvais B, et al. Disseminated microsporidiosis due to *Septata intestinalis* in patients with AIDS: clinical features and response to albendazole therapy. *J Infect Dis*. 1995;171:245–249.
191. Molina JM, Tourneur M, Sarfati C, et al. Fumagillin treatment of intestinal microsporidiosis. *N Engl J Med*. 2002;346:1963–1969.
192. Molina JM, Goguel J, Sarfati C, et al. Trial of oral fumagillin for the treatment of intestinal microsporidiosis in patients with HIV infection; ANRS 054 Study Group: Agence Nationale de Recherche sur le SIDA. *AIDS*. 2000;14:1341–1348.
193. Bergquist NR, Stintzing G, Smedman L, et al. Diagnosis of encephalitozoonosis in man by serological tests. *BMJ*. 1984;288:902.
194. Aldras AM, Orenstein JM, Kotler DP, et al. Detection of microsporidia by indirect immunofluorescence antibody test using polyclonal and monoclonal antibodies. *J Clin Microbiol*. 1994;32:608–612.
195. Weiss LM, Cali A, Levee E, et al. Diagnosis of *Encephalitozoon cuniculi* infection by Western blot and the use of cross-reactive antigens for the possible detection of microsporidiosis in humans. *Am J Trop Med Hyg*. 1992;47:456–462.
196. Singh M, Kane GJ, Mackinlay L, et al. Detection of antibodies to *Nosema cuniculi* (Protozoa: Microscoporidia) in human and animal sera by the indirect fluorescent antibody technique. *Southeast Asian J Trop Med Public Health*. 1982;13:110–113.
197. WHO parasitic diseases surveillance: antibody to *Encephalitozoon cuniculi* in man. *Wkly Epidemiol Rec*. 1983;58:30.
198. Bergquist R, Morfeldt-Mansson L, Pehrson PO, et al. Antibody against *Encephalitozoon cuniculi* in Swedish homosexual men. *Scand J Infect Dis*. 1984;16:389–391.
199. Van Gool T, Vetter JC, Weinmayr B, et al. High seroprevalence of *Encephalitozoon* species in immunocompetent subjects. *J Infect Dis*. 1997;175:1020–1024.
200. Pospisilova Z, Ditrich O, Stankova M, et al. Parasitic opportunistic infections in Czech HIV-infected patients: a prospective study. *Cent Eur J Public Health*. 1997;5:208–213.
201. Cislakova L, Prokopcakova H, Stef'kovic M, et al. *Encephalitozoon cuniculi*—clinical and epidemiologic significance: results of a preliminary serologic study in humans. *Epidemiol Mikrobiol Immunol*. 1997;46:30–33.
202. Didier ES, Varner PW, Didier PJ, et al. Experimental microsporidiosis in immunocompetent and immunodeficient mice and monkeys. *Folia Parasitol*. 1994;41:1–11.
203. Ghosh K, Weiss LM. T cell response and persistence of the microsporidia. *FEMS Microbiol Rev*. 2012;36:748–760.
204. Sak B, Kotková M, Hlásková L, et al. Limited effect of adaptive immune response to control encephalitozoonosis. *Parasite Immunol*. 2017;39:e12496.
205. Koudela B, Vitovec J, Kucerova Z, et al. The severe combined immunodeficient mouse as a model for *Encephalitozoon cuniculi* microsporidiosis. *Folia Parasitol (Praha)*. 1993;40:279–286.
206. Schmidt EC, Shadduck JA. Mechanisms of resistance to the intracellular protozoan *Encephalitozoon cuniculi* in mice. *J Immunol*. 1984;133:2712–2719.
207. Hermanek J, Koudela B, Kucerova Z, et al. Prophylactic and therapeutic immune reconstitution of SCID mice infected with *Encephalitozoon cuniculi*. *Folia Parasitol (Praha)*. 1993;40:287–291.
208. Didier ES, Shadduck JA. IFN-gamma and LPS induce murine macrophages to kill *Encephalitozoon cuniculi* in vitro. *J Eukaryot Microbiol*. 1994;41:34S.
209. Didier ES. Reactive nitrogen intermediates implicated in the inhibition of *Encephalitozoon cuniculi* (phylum Microspora) replication in murine peritoneal macrophages. *Parasite Immunol*. 1995;17:405–412.
210. Didier ES, Bowers LC, Martin AD, et al. Reactive nitrogen and oxygen species, and iron sequestration contribute to macrophage-mediated control of Encephalitozoon cuniculi (phylum Microsporidia) infection in vitro and in vivo. *Microbes Infect*. 2010;12:1244–1251.
211. Khan IA, Moretto M. Role of gamma interferon in cellular immune response against murine *Encephalitozoon cuniculi* infection. *Infect Immun*. 1999;67:1887–1893.
212. Bywater JE, Kellett BS. Humoral immune response to natural infection with Encephalitozoon cuniculi in rabbits. *Lab Anim*. 1979;13:293–297.
213. Enriquez FJ, Ditrich O, Palting JD, et al. Simple diagnosis of *Encephalitozoon* sp. microsporidial infections by using a pan-specific antiexospore monoclonal antibody. *J Clin Microbiol*. 1997;35:724–729.
214. Furuya K, Omura M, Kudo S, et al. Recognition profiles of microsporidian *Encephalitozoon cuniculi* polar tube protein 1 with human immunoglobulin M antibodies. *Parasite Immunol*. 2008;30:13–21.
215. Shtrichman R, Samuel CE. The role of gamma interferon in antimicrobial immunity. *Curr Opin Microbiol*. 2001;4:251–259.
216. Achbarou A, Ombrouck C, Gneragbe T, et al. Experimental model for human intestinal microsporidiosis in interferon gamma receptor knockout mice infected by *Encephalitozoon intestinalis*. *Parasite Immunol*. 1996;18:387–392.
217. Khan IA, Schwartzman JD, Kasper LH, et al. CD8+ CTLs are essential for protective immunity against *Encephalitozoon cuniculi* infection. *J Immunol*. 1999;162:6086–6091.
218. Moretto MM, Lawlor EM, Khan IA. Lack of interleukin-12 in p40-deficient mice leads to poor CD8+ T-cell immunity against *Encephalitozoon cuniculi* infection. *Infect Immun*. 2010;78:2505–2511.
219. Wong P, Pamer EG. CD8 T cell responses to infectious pathogens. *Annu Rev Immunol*. 2003;21:29–70.
220. Moretto M, Weiss LM, Khan IA. Induction of a rapid and strong antigen-specific intraepithelial lymphocyte response during oral *Encephalitozoon cuniculi* infection. *J Immunol*. 2004;172:4402–4409.
221. Moretto MM, Khan IA, Weiss LM. Gastrointestinal cell mediated immunity and the microsporidia. *PLoS Pathog*. 2012;8:e1002775.
222. Moretto MM, Weiss LM, Combe CL, et al. INF gamma-producing dendritic cells are important for priming of the gut intraepithelial lymphocyte response against intracellular parasitic infection. *J Immunol*. 2007;179:2485–2492.
223. Lawlor EM, Moretto MM, Khan IA. Optimal CD8 T-cell response against *Encephalitozoon cuniculi* is mediated by Toll-like receptor 4 upregulation by dendritic cells. *Infect Immun*. 2010;78:3097–3102.
224. Fischer J, Suire C, Hale-Donze H. Toll-like receptor 2 recognition of the microsporidia *Encephalitozoon* spp. induces nuclear translocation of NF-kappaB and subsequent inflammatory responses. *Infect Immun*. 2008;76:4737–4744.
225. Gigley JP, Khan IA. Plasmacytoid DC from aged mice down-regulate CD8 T cell responses by inhibiting cDC maturation after *Encephalitozoon cuniculi* infection. *PLoS ONE*. 2011;6:e20838.
226. Conteas CN, Berlin OG, Speck CE, et al. Modification of the clinical course of intestinal microsporidiosis in acquired immunodeficiency syndrome patients by immune status and anti-human immunodeficiency virus therapy. *Am J Trop Med Hyg*. 1998;58:555–558.
227. Goguel J, Katlama C, Sarfati C, et al. Remission of AIDS-associated intestinal microsporidiosis with highly active antiretroviral therapy. *AIDS*. 1997;11:1658–1659.
228. Foudraine NA, Weverling GJ, van Gool T, et al. Improvement of chronic diarrhoea in patients with advanced HIV-1 infection during potent antiretroviral therapy. *AIDS*. 1998;12:35–41.
229. Terada S, Reddy KR, Jeffers LJ, et al. Microsporidan hepatitis in the acquired immunodeficiency syndrome. *Ann Intern Med*. 1987;107:61–62.
230. Schwartz DA, Anderson DC, Klumpp SA, et al. Ultrastructure of atypical (teratoid) sporogonial stages of *Enterocytozoon bieneusi* (Microsporidia) in naturally infected rhesus monkeys (Macaca mulatta). *Arch Pathol Lab Med*. 1998;122:423–429.
231. Orenstein JM, Tenner M, Kotler DP. Localization of infection by the microsporidian *Enterocytozoon bieneusi*

in the gastrointestinal tract of AIDS patients with diarrhea. *AIDS*. 1992;6:195–197.

232. Pol S, Romana CA, Richard S, et al. Microsporidia infection in patients with the human immunodeficiency virus and unexplained cholangitis. *N Engl J Med*. 1993;328:95–99.
233. Orenstein JM. Diagnostic pathology of microsporidiosis. *Ultrastruct Pathol*. 2003;27:141–149.
234. Schwartz DA, Sobottka I, Leitch GJ, et al. Pathology of microsporidiosis: emerging parasitic infections in patients with acquired immunodeficiency syndrome. *Arch Pathol Lab Med*. 1996;120:173–188.
235. Schwartz DA, Abou-Elella A, Wilcox CM, et al. The presence of *Enterocytozoon bieneusi* spores in the lamina propria of small bowel biopsies with no evidence of disseminated microsporidiosis. Enteric Opportunistic Infections Working Group. *Arch Pathol Lab Med*. 1995;119:424–428.
236. Orenstein JM, Dieterich DT, Kotler DP. Systemic dissemination by a newly recognized intestinal microsporidia species in AIDS. *AIDS*. 1992;6:1143–1150.
237. Soule JB, Halverson AL, Becker RB, et al. A patient with acquired immunodeficiency syndrome and untreated *Encephalitozoon (Septata) intestinalis* microsporidiosis leading to small bowel perforation: response to albendazole. *Arch Pathol Lab Med*. 1997;121:880–887.
238. Tanveer N, Barman S. *Microsporidium* infection and perforation peritonitis: a rare association. *Indian J Sex Transm Dis*. 2015;36:188–191.
239. Zender HO, Arrigoni E, Eckert J, et al. A case of *Encephalitozoon cuniculi* peritonitis in a patient with AIDS. *Am J Clin Pathol*. 1989;92:352–356.
240. Gunnarsson G, Hurlbut D, DeGirolami PC, et al. Multiorgan microsporidiosis: report of five cases and review. *Clin Infect Dis*. 1995;21:37–44.
241. Guerard A, Rabodonirina M, Cotte L, et al. Intestinal microsporidiosis occurring in two renal transplant recipients treated with mycophenolate mofetil. *Transplantation*. 1999;68:699–707.
242. Latib MA, Pascoe MD, Duffield MS, et al. Microsporidiosis in the graft of a renal transplant recipient. *Transpl Int*. 2001;14:274–277.
243. Torres G, Izquierdo F, Capó V, et al. Genital microsporidiosis in women with AIDS: a post-mortem study. *Rev Iberoam Micol*. 2013;30:47–50.
244. Mertens RB, Didier ES, Fishbein MC, et al. *Encephalitozoon cuniculi* microsporidiosis: infection of the brain, heart, kidneys, trachea, adrenal glands, and urinary bladder in a patient with AIDS. *Mod Pathol*. 1997;10:68–77.
245. Pariyakanok L, Jongwutiwes S. Keratitis caused by *Trachipleistophora anthropopthera*. *J Infect*. 2005;51:325–328.
246. Cali A, Meisler DM, Rutherford I, et al. Corneal microsporidiosis in a patient with AIDS. *Am J Trop Med Hyg*. 1991;44:463–468.
247. Joseph J, Sharma S, Murthy SI, et al. Microsporidial keratitis in India: 16S rRNA gene-based PCR assay for diagnosis and species identification of microsporidia in clinical samples. *Invest Ophthalmol Vis Sci*. 2006;47:4468–4473.
248. Pradhan S, Mascarenhas J, Srinivasan M. Microsporidial stromal keratitis masquerading as acute graft rejection. *Cornea*. 2015;34:353–354.
249. Filho MM, Ribeiro HB, Paula LJ, et al. Images in cardiovascular medicine. Endocarditis secondary to microsporidia: giant vegetation in a pacemaker user. *Circulation*. 2009;119:e386–e388. Erratum in: Circulation. 2010;121:e9.
250. Coyle CM, Weiss LM, Rhodes LV 3rd, et al. Fatal myositis due to the microsporidian *Brachiola algerae*, a mosquito pathogen. *N Engl J Med*. 2004;351:42–47.
251. Patel AK, Patel KK, Chickabasaviah YT, et al. Microsporidial polymyositis in human immunodeficiency virus-infected patients, a rare life-threatening opportunistic infection: clinical suspicion, diagnosis, and management in resource-limited settings. *Muscle Nerve*. 2015;51:775–780.
252. Grau A, Valls ME, Williams JE, et al. Myositis caused by *Pleistophora* in a patient with AIDS. *Med Clin (Barc)*. 1996;107:779–781.
253. Ledford DK, Overman MD, Gonzalvo A, et al. Microsporidiosis myositis in a patient with the acquired immunodeficiency syndrome. *Ann Intern Med*. 1985;102:628–630.
254. Schwartz DA, Visvesvara GS, Leitch GJ, et al. Pathology of symptomatic microsporidial *(Encephalitozoon hellem)* bronchiolitis in the acquired immunodeficiency syndrome: a new respiratory pathogen diagnosed from lung biopsy, bronchoalveolar lavage, sputum, and tissue culture. *Hum Pathol*. 1993;24:937–943.
255. Didier ES, Rogers LB, Orenstein JM, et al. Characterization of *Encephalitozoon (Septata) intestinalis* isolates cultured from nasal mucosa and bronchoalveolar lavage fluids of two AIDS patients. *J Eukaryot Microbiol*. 1996;43:34–43.
256. Dunand VA, Hammer SM, Rossi R, et al. Parasitic sinusitis and otitis in patients infected with human immunodeficiency virus: report of five cases and review. *Clin Infect Dis*. 1997;25:267–272.
257. Franzen C, Muller A, Salzberger B, et al. Chronic rhinosinusitis in patients with AIDS: potential role of microsporidia. *AIDS*. 1996;10:687–688.
258. Gritz DC, Holsclaw DS, Neger RE, et al. Ocular and sinus microsporidial infection cured with systemic albendazole. *Am J Ophthalmol*. 1997;124:241–243.
259. Josephson GD, Sarlin J, Reidy J, et al. Microsporidial rhinosinusitis: is this the next pathogen to infect the sinuses of the immunocompromised host? *Otolaryngol Head Neck Surg*. 1996;114:137–139.
260. Moss RB, Beaudet LM, Wenig BM, et al. *Microsporidium*-associated sinusitis. *Ear Nose Throat J*. 1997;76:95–101.
261. Pedro-de-Lelis FJ, Sabater-Marco V, Herrera-Ballester A. Necrotizing maxillary sinus mucormycosis related to candidiasis and microsporidiosis in an AIDS patient. *AIDS*. 1995;9:1386–1388.
262. Degroote MA, Visvesvara G, Wilson ML, et al. Polymerase chain reaction and culture confirmation of disseminated *Encephalitozoon cuniculi* in a patient with AIDS: successful therapy with albendazole. *J Infect Dis*. 1995;171:1375–1378.
263. Schwartz DA, Bryan RT, Hewanlowe KO, et al. Disseminated microsporidiosis *(Encephalitozoon hellem)* and acquired immunodeficiency syndrome: autopsy evidence for respiratory acquisition. *Arch Pathol Lab Med*. 1992;116:660–668.
264. Franzen C, Mueller A, Salzberger B, et al. Chronic rhinosinusitis in patients with AIDS: potential role of microsporidia. *AIDS*. 1996;10:687–688.
265. Del Aguila C, Lopez-Velez R, Fenoy S, et al. Identification of *Enterocytozoon bieneusi* spores in respiratory samples from an AIDS patient with a 2-year history of intestinal microsporidiosis. *J Clin Microbiol*. 1997;35:1862–1866.
266. Weber R, Kuster H, Keller R, et al. Pulmonary and intestinal microsporidiosis in a patient with the acquired immunodeficiency syndrome. *Am Rev Respir Dis*. 1992;146:1603–1605.
267. Hartskeerl RA, Schuitema AR, van Gool T, et al. Genetic evidence for the occurrence of extra-intestinal *Enterocytozoon bieneusi* infections. *Nucleic Acids Res*. 1993;21:4150.
268. Visvesvara GS, Belloso M, Moura H, et al. Isolation of *Nosema algerae* from the cornea of an immunocompetent patient. *J Eukaryot Microbiol*. 1999;46:10S.
269. Kester KE, Visvesara GS, McEvoy P. Organism responsible for nodular cutaneous microsporidiosis in a patient with AIDS. *Ann Intern Med*. 2000;133:925.
270. Kester KE, Turiansky GW, McEvoy PL. Nodular cutaneous microsporidiosis in a patient with AIDS and successful treatment with long-term oral clindamycin therapy. *Ann Intern Med*. 1998;128:911–914.
271. Levaditi C, Nicolau S, Schoen R. Nouvelles donnees sur L'*Encephalitozoon cuniculi*. *C R Soc Biol*. 1923;89:1157–1162.
272. Margileth AM, Strano AJ, Chandra R, et al. Disseminated nosematosis in an immunologically compromised infant. *Arch Pathol*. 1973;95:145–150.
273. Lopez-Velez R, Turrientes MC, Garron C, et al. Microsporidiosis in travelers with diarrhea from the tropics. *J Travel Med*. 1999;6:223–227.
274. Muller A, Bialek R, Kamper A, et al. Detection of microsporidia in travelers with diarrhea. *J Clin Microbiol*. 2001;39:1630–1632.
275. Desportes-Livage I, Doumbo O, Pichard E, et al. Microsporidiosis in HIV-seronegative patients in Mali. *Trans R Soc Trop Med Hyg*. 1998;92:423–424.
276. Field AS, Paik JY, Stark D, et al. Myositis due to the microsporidian *Anncaliia (Brachiola) algerae* in a lung transplant recipient. *Transpl Infect Dis*. 2012;14:169–176.
277. Cali A, Neafie R, Weiss LM, et al. Human vocal cord infection with the Microsporidium *Anncaliia algerae*. *J Eukaryot Microbiol*. 2010;57:562–567.
278. Goetz M, Eichenlaub S, Pape GR, et al. Chronic diarrhea as a result of intestinal microsporidiosis in a liver transplant recipient. *Transplantation*. 2001;71:334–337.
279. Kelkar R, Sastry PS, Kulkarni SS, et al. Pulmonary microsporidial infection in a patient with CML undergoing allogeneic marrow transplant. *Bone Marrow Transplant*. 1997;19:179–182.
280. Ladapo TA, Nourse P, Pillay K, et al. Microsporidiosis in pediatric renal transplant patients in Cape Town, South Africa: two case reports. *Pediatr Transplant*. 2014;18:E220–E226.
281. Kicia M, Wesolowska M, Jakuszko K, et al. Concurrent infection of the urinary tract with *Encephalitozoon cuniculi* and *Enterocytozoon bieneusi* in a renal transplant recipient. *J Clin Microbiol*. 2014;52:1780–1782.
282. Bukreyeva I, Angoulvant A, Bendib I, et al. *Enterocytozoon bieneusi* microsporidiosis in stem cell transplant recipients treated with fumagillin. *Emeg Infect Dis*. 2017;23:1039–1041.
283. Nagpal A, Pritt BS, Lorenz EC, et al. Disseminated microsporidiosis in a renal transplant recipient: case report and review of the literature. *Transpl Infect Dis*. 2013;15:526–532.
284. Nevez G, Le Gal S, Quinio D, et al. *Encephalitozoon hellem* in a patient with CD4+ T-cell prolymphocytic leukemia: case report and genomic identification. *Diagn Microbiol Infect Dis*. 2015;83:245–247.
285. Godron A, Accoceberry I, Couret A, et al. Intestinal microsporidiosis due to *Enterocytozoon bieneusi* in a pediatric kidney transplant recipient successfully treated with fumagillin. *Transplantation*. 2013;96:e66–e67.
286. Desoubeaux G, Maakaroun-Vermesse Z, Lier C, et al. Successful treatment with fumagillin of the first pediatric case of digestive microsporidiosis in a liver-kidney transplant. *Transpl Infect Dis*. 2013;15:E250–E259.
287. Aikawa NE, Twardowsky Ade O, Carvalho JF, et al. Intestinal microsporidiosis: a hidden risk in rheumatic disease patients undergoing anti-tumor necrosis factor therapy combined with disease-modifying anti-rheumatic drugs? *Clinics (Sao Paulo)*. 2011;66:1171–1175.
288. Pinnolis M, Egbert PR, Font RL, et al. Nosematosis of the cornea: case report, including electron microscopic studies. *Arch Ophthalmol*. 1981;99:1044–1047.
289. Ashton N, Wirasinha PA. Encephalitozoonosis (nosematosis) of the cornea. *Br J Ophthalmol*. 1973;57:669–674.
290. Cali A, Meisler DM, Lowder CY, et al. Corneal microsporidioses: characterization and identification. *J Protozool*. 1991;38:215S–217S.
291. Sood AB, Debiec MR, Yeh S, et al. Microsporidial stromal keratitis and endophthalmitis in an immunocompetent patient. *J Ophthalmic Inflam Infect*. 2016;6:30.
292. Davis RM, Font RL, Keisler MS, et al. Corneal microsporidiosis: a case report including ultrastructural observations. *Ophthalmology*. 1990;97:953–957.
293. Khandelwal SS, Woodward MA, Hall T, et al. Treatment of microsporidia keratitis with topical voriconazole monotherapy. *Arch Ophthalmol*. 2011;129:509–510.
294. Sanjay S. Clinical trial of 0.02% polyhexamethylene biguanide versus placebo in the treatment of microsporidial keratoconjunctivitis. *Am J Ophthalmol*. 2011;151:183. author reply 183.
295. Das S, Sharma S, Sahu SK, et al. Diagnosis, clinical features and treatment outcome of microsporidial keratoconjunctivitis. *Br J Ophthalmol*. 2012;96:793–795.
296. Sharma S, Das S, Joseph J, et al. Microsporidial keratitis: need for increased awareness. *Surv Ophthalmol*. 2011;56:1–22.
297. Fan NW, Lin PY, Chen TL, et al. Treatment of microsporidial keratoconjunctivitis with repeated corneal swabbing. *Am J Ophthalmol*. 2012;154:927–933.e1.
298. Rauz S, Tuft S, Dart JK, et al. Ultrastructural examination of two cases of stromal microsporidial keratitis. *J Med Microbiol*. 2004;53:775–781.
299. Ditrich O, Chrdle A, Sak B, et al. *Encephalitozoon cuniculi* genotype I as a causative agent of brain abscess in an immunocompetent patient. *J Clin Microbiol*. 2011;49:2769–2771.
300. Watts MR, Chan RC, Cheong EY, et al. *Anncaliia algerae* microsporidial myositis. *Emerg Infect Dis*. 2014;20:185–191.
301. Boileau M, Ferreira J, Ahmed I, et al. Successful treatment of disseminated *Anncaliia algerae* microsporidial infection with combination fumagillin and albendazole. *Open Forum Infect Dis*. 2016;3:ofw158.
302. Kicia M, Kopacz S, Wesolowska M, et al Disseminated *Encephalitozoon cuniculi* infection in patients with hip implant loosening. International Workshops on Opportunistic Protists-14, Cincinnati, Ohio, 2017:1744.
303. Loignon M, Labrecque LG, Bard C, et al. Cerebral microsporidiosis manifesting as progressive multifocal leukoencephalopathy in an HIV-infected individual—a case report. *AIDS Res Ther*. 2014;11:20.
304. Weber R, Muller A, Spycher MA, et al. Intestinal *Enterocytozoon bieneusi* microsporidiosis in an HIV-infected patient: diagnosis by ileo-colonoscopic biopsies and long-term follow up. *Clin Investig*. 1992;70:1019–1023.
305. Rijpstra AC, Canning EU, Van Ketel RJ, et al. Use of light microscopy to diagnose small-intestinal microsporidiosis in patients with AIDS. *J Infect Dis*. 1988;157:827–831.
306. Molina JM, Sarfati C, Beauvais B, et al. Intestinal microsporidiosis in human immunodeficiency virus-infected patients with chronic unexplained diarrhea: prevalence and clinical and biologic features. *J Infect Dis*. 1993;167:217–221.

307. Hewan-Lowe K, Furlong B, Sims M, et al. Coinfection with *Giardia lamblia* and *Enterocytozoon bieneusi* in a patient with acquired immunodeficiency syndrome and chronic diarrhea. *Arch Pathol Lab Med*. 1997;121:417–422.
308. Beaugerie L, Teilhac MF, Deluol AM, et al. Cholangiopathy associated with microsporidia infection of the common bile duct mucosa in a patient with HIV infection. *Ann Intern Med*. 1992;117:401–402.
309. Hartskeerl RA, van Gool T, Schuitema ARJ, et al. Genetic and immunological characterization of the microsporidian *Septata intestinalis* Cali, Kotler and Orenstein, 1993: reclassification to *Encephalitozoon intestinalis*. *Parasitology*. 1995;110:277–285.
310. Didier ES, Didier PJ, Snowden KF, et al. Microsporidiosis in mammals. *Microbes Infect*. 2000;2:709–720.
311. Schwartz DA, Visvesvara GS, Leitch GJ, et al. Pathology of symptomatic microsporidial *(Encephalitozoon hellem)* bronchiolitis in the acquired immunodeficiency syndrome: a new respiratory pathogen diagnosed from lung biopsy, bronchoalveolar lavage, sputum, and tissue culture. *Hum Pathol*. 1993;24:937–943.
312. Van Gool T, Canning EU, Gilis H, et al. *Septata intestinalis* frequently isolated from stool of AIDS patients with a new cultivation method. *Parasitology*. 1994;109:281–289.
313. Willson R, Harrington R, Stewart B, et al. Human immunodeficiency virus 1-associated necrotizing cholangitis caused by infection with *Septata intestinalis*. *Gastroenterology*. 1995;108:247–251.
314. Belcher JW Jr, Guttenberg SA, Schmookler BM. Microsporidiosis of the mandible in a patient with acquired immunodeficiency syndrome. *J Oral Maxillofac Surg*. 1997;55:424–426.
315. Dore GJ, Marriott DJ, Hing MC, et al. Disseminated microsporidiosis due to *Septata intestinalis* in nine patients infected with the human immunodeficiency virus: response to therapy with albendazole. *Clin Infect Dis*. 1995;21:70–76.
316. Weber R, Sauer B, Spycher MA, et al. Detection of *Septata intestinalis* in stool specimens and coprodiagnostic monitoring of successful treatment with albendazole. *Clin Infect Dis*. 1994;19:342–345.
317. De Groote MA, Visvesvara G, Wilson ML, et al. Polymerase chain reaction and culture confirmation of disseminated *Encephalitozoon cuniculi* in a patient with AIDS: successful therapy with albendazole. *J Infect Dis*. 1995;171:1375–1378.
318. Weber R, Kuster H, Visvesvara GS, et al. Disseminated microsporidiosis due to *Encephalitozoon hellem:* pulmonary colonization, microhematuria, and mild conjunctivitis in a patient with AIDS. *Clin Infect Dis*. 1993;17:415–419.
319. Visvesvara GS, Leitch GJ, da Silva AJ, et al. Polyclonal and monoclonal antibody and PCR-amplified small-subunit rRNA identification of a microsporidian, *Encephalitozoon hellem,* isolated from an AIDS patient with disseminated infection. *J Clin Microbiol*. 1994;32:2760–2768.
320. Lowder CY, Meisler DM, McMahon JT, et al. Microsporidia infection of the cornea in a man seropositive for human immunodeficiency virus. *Am J Ophthalmol*. 1990;109:242–244.
321. Lacey CJN, Clarke AMT, Fraser P, et al. Chronic microsporidian infection of the nasal mucosae, sinuses and conjunctivae in HIV disease. *Genitourin Med*. 1992;68:179–181.
322. Franzen C, Schwartz DA, Visvesvara GS, et al. Immunologically confirmed disseminated, asymptomatic *Encephalitozoon cuniculi* infection of the gastrointestinal tract in a patient with AIDS. *Clin Infect Dis*. 1995;21:1480–1484.
323. Macher AR, Neafie R, Angritt P, et al. Microsporidia myositis and the acquired immunodeficiency syndrome (AIDS): a four-year followup. *Ann Intern Med*. 1988;109:343–344.
324. Weber R, Bryan RT, Owen RL, et al. Improved light-microscopal detection of microsporidia spores in stool and duodenal aspirates. *N Engl J Med*. 1992;326:161–166.
325. Vavra J, Dahbiova R, Hollister WS, et al. Staining of microsporidian spores by optical brighteners with remarks on the use of brighteners for the diagnosis of AIDS-associated human microsporidiosis. *Folia Parasitol (Praha)*. 1993;40:267–272.
326. Van Gool T, Snijders F, Reiss P, et al. Diagnosis of intestinal and disseminated microsporidial infections in patients with HIV by a new rapid fluorescence technique. *J Clin Pathol*. 1993;46:694–699.
327. Ryan NJ, Sutherland G, Coughlan K, et al. A new trichrome-blue stain for detection of microsporidial species in urine, stool, and nasopharyngeal specimens. *J Clin Microbiol*. 1993;31:3264–3269.
328. Kokoskin E, Gyorkos TW, Camus A, et al. Modified technique for efficient detection of microsporidia. *J Clin Microbiol*. 1994;32:1074–1075.
329. Moura H, Schwartz DA, Bornay-Llinares F, et al. A new and improved "quick-hot Gram-chromotrope" technique that differentially stains microsporidian spores in clinical samples, including paraffin-embedded tissue sections. *Arch Pathol Lab Med*. 1997;121:888–893.
330. Didier ES, Orenstein JM, Aldras A, et al. Comparison of three staining methods for detecting microsporidia in fluids. *J Clin Microbiol*. 1995;33:3138–3145.
331. DeGirolami PC, Ezratty CR, Desai G, et al. Diagnosis of intestinal microsporidiosis by examination of stool and duodenal aspirate with Weber's modified trichrome and Uvitex 2B strains. *J Clin Microbiol*. 1995;33:805–810.
332. Field AS, Marriott DJ, Hing MC. The Warthin-Starry stain in the diagnosis of small intestinal microsporidiosis in HIV-infected patients. *Folia Parasitol (Praha)*. 1993;40:261–266.
333. Field AS. Light microscopic and electron microscopic diagnosis of gastrointestinal opportunistic infections in HIV-positive patients. *Pathology*. 2002;34:21–35.
334. Peterson TS, Spitsbergen JM, Feist SW, et al. Luna stain, an improved selective stain for detection of microsporidian spores in histologic sections. *Dis Aquat Organ*. 2011;95:175–180.
335. Levine DJ, Riley DJ, Jorgensen JH, et al. Key diagnostic features of granulomatous interstitial nephritis due to *Encephalitozoon cuniculi* in a lung transplant recipient. *Am J Surg Pathol*. 2013;37:447–452.
336. Zierdt CH, Gill VJ, Zierdt WS. Detection of microsporidian spores in clinical samples by indirect fluorescent-antibody assay using whole-cell antisera to *Encephalitozoon cuniculi* and *Encephalitozoon hellem*. *J Clin Microbiol*. 1993;31:3071–3074.
337. Croppo GP, Visvesvara GS, Leitch GJ, et al. Identification of the microsporidian Encephalitozoon hellem using immunoglobulin G monoclonal antibodies. *Arch Pathol Lab Med*. 1998;122:182–186.
338. Beckers PJ, Derks GJ, Gool T, et al. *Encephalitozoon intestinalis*-specific monoclonal antibodies for laboratory diagnosis of microsporidiosis. *J Clin Microbiol*. 1996;34:282–285.
339. Accoceberry I, Thellier M, Desportes-Livage I, et al. Production of monoclonal antibodies directed against the microsporidium *Enterocytozoon bieneusi*. *J Clin Microbiol*. 1999;37:4107–4112.
340. Zhang Q, Singh I, Sheroan A, et al. Production and characterization of monoclonal antibodies against *Enterocytozoon bieneusi* purified from rhesus macaques. *Infect Immun*. 1994;73:5166–5172.
341. Sheoran AS, Feng X, Singh I, et al. Monoclonal antibodies against *Enterocytozoon bieneusi* of human origin. *Clin Diagn Lab Immunol*. 2005;12:1109–1113.
342. Singh I, Sheroran AS, Zhang Q, et al. Sensitivity and specificity of a monoclonal antibody-based fluorescence assay for detecting *Enterocytozoon bieneusi* spores in feces of simian immunodeficiency virus-infected macaques. *Clin Diagn Lab Immunol*. 2005;12:1141–1144.
343. Visvesvara GS. In vitro cultivation of microsporidia of clinical importance. *Clin Microbiol Rev*. 2002;15:401–413.
344. Visvesvara GS, Leitch GJ, Pieniazek NJ, et al. Short-term in vitro culture and molecular analysis of the microsporidian, *Enterocytozoon bieneusi*. *J Eukaryot Microbiol*. 1995;42:506–510.
345. Visvesvara GS, Leitch GJ, Wallace S, et al. Adenovirus masquerading as microsporidia. *J Parasitol*. 1996;82:316–319.
346. Tzipori S, Carville A, Widmer G, et al. Transmission and establishment of a persistent infection of *Enterocytozoon bieneusi,* derived from a human with AIDS, in simian immunodeficiency virus-infected rhesus monkeys. *J Infect Dis*. 1997;175:1016–1020.
347. Feng X, Akiyoshi DE, Sheroran A, et al. Serial propagation of the microsporidian *Enterocytozoon bieneusi* of human origin in immunocompromised rodents. *Infect Immun*. 2006;74:4424–4429.
348. Didier ES. Immunology of microsporidiosis. *Contrib Microbiol*. 2000;6:193–208.
349. Ghosh K, Weiss LM. Molecular diagnostic tests for microsporidia. *Interdiscip Perspect Infect Dis*. 2009;2009:926521.
350. Katzwinkel-Wladarsch S, Deplazes P, Weber R, et al. Comparison of polymerase chain reaction with light microscopy for detection of microsporidia in clinical specimens. *Eur J Clin Microbiol Infect Dis*. 1997;16:7–10.
351. Fedorko DP, Nelson NA, Cartwright CP. Identification of microsporidia in stool specimens by using PCR and restriction endonucleases. *J Clin Microbiol*. 1995;33:1739–1741.
352. Ombrouck C, Ciceron L, Biligui S, et al. Specific PCR assay for direct detection of intestinal microsporidia *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* in fecal specimens from human immunodeficiency virus-infected patients. *J Clin Microbiol*. 1997;35:652–655.
353. Maggi P, Larocca AM, Quarto M, et al. Effect of antiretroviral therapy on cryptosporidiosis and microsporidiosis in patients infected with human immunodeficiency virus type 1. *Eur J Clin Microbiol Infect Dis*. 2000;19:213–217.
354. Martins SA, Muccioli C, Belfort R Jr, et al. Resolution of microsporidial keratoconjunctivitis in an AIDS patient treated with highly active antiretroviral therapy. *Am J Ophthalmol*. 2001;131:378–379.
355. Miao YM, Awad-El-Kariem FM, Franzen C, et al. Eradication of cryptosporidia and microsporidia following successful anti-retroviral therapy. *J Acquir Immune Defic Syndr*. 2000;25:124–129.
356. Sriaroon C, Mayer CA, Chen L, et al. Diffuse intra-abdominal granulomatous seeding as a manifestation of immune reconstitution inflammatory syndrome associated with microsporidiosis in a patient with HIV. *AIDS Patient Care STDS*. 2008;22:611–612.
357. Beauvais B, Sarfati C, Challier S, et al. In vitro model to assess effect of antimicrobial agents on *Encephalitozoon cuniculi*. *Antimicrob Agents Chemother*. 1994;38:2440–2448.
358. Franssen FF, Lumeij JT, van Knapen F. Susceptibility of *Encephalitozoon cuniculi* to several drugs in vitro. *Antimicrob Agents Chemother*. 1995;39:1265–1268.
359. Costa SF, Weiss LM. Drug treatment of microsporidiosis. *Drug Resist Update*. 2000;3:384–399.
360. Didier ES. Effects of albendazole, fumagillin, and TNP-470 on microsporidial replication in vitro. *Antimicrob Agents Chemother*. 1997;41:1541–1546.
361. Li J, Katiyar SK, Hamelin A, et al. Tubulin genes from AIDS-associated microsporidia and implications for phylogeny and benzimidazole sensitivity. *Mol Biochem Parasitol*. 1996;78:289–295.
362. Akiyoshi DE, Weiss LM, Feng X, et al. Analysis of the beta-tubulin genes from *Enterocytozoon bieneusi* isolates form a human and rhesus macaque. *J Eukaryot Microbiol*. 2007;54:38–41.
363. Franzen C, Salzberger B. Analysis of the beta-tubulin gene from *Vittaforma corneae* suggests benzimidazole resistance. *Antimicrob Agents Chemother*. 2008;52:790–793.
364. Shah V, Marino C, Altice FL. Albendazole-induced pseudomembranous colitis. *Am J Gastroenterol*. 1996;91:1453–1454.
365. Lecuit M, Oksenhendler E, Sarfati C. Use of albendazole for disseminated microsporidian infection in a patient with AIDS. *J Infect Dis*. 1994;19:332–333.
366. Visvesvara GS, Leitch GJ, Dasilva AJ, et al. Polyclonal and monoclonal antibody and PCR-amplified small-subunit rRNA identification of a microsporidian, *Encephalitozoon hellem,* isolated from an AIDS patient with disseminated infection. *J Clin Microbiol*. 1994;32:2760–2768.
367. Corcoran GD, Isaacson JR, Daniels C, et al. Urethritis associated with disseminated microsporidiosis: clinical response to albendazole. *Clin Infect Dis*. 1996;22:592–593.
368. Aarons EJ, Woodrow D, Hollister WS, et al. Reversible renal failure caused by a microsporidian infection. *AIDS*. 1994;8:1119–1121.
369. Blanshard C, Peacock C, Ellis D, et al Treatment of intestinal microsporidiosis with albendazole. In: Proceedings of the VII International Conference on AIDS: Science Challenging AIDS, vol 1. Clinical Science and Trials. Florence, Italy, 1991.
370. Dieterich DT, Lew EA, Kotler DP, et al. Treatment with albendazole for intestinal disease due to *Enterocytozoon bieneusi* in patients with AIDS. *J Infect Dis*. 1994;169:178–183.
371. Molina JM, Chastang C, Goguel J, et al. Albendazole for treatment and prophylaxis of microsporidiosis due to *Encephalitozoon intestinalis* in patients with AIDS: a randomized double-blind controlled trial. *J Infect Dis*. 1998;177:1373–1377.
372. Sobottka I, Albrecht H, Schafer H, et al. Disseminated *Encephalitozoon (Septata) intestinalis* infection in a patient with AIDS: novel diagnostic approaches and autopsy-confirmed parasitological cure following treatment with albendazole. *J Clin Microbiol*. 1995;33:2948–2952.
373. Blanshard C, Ellis DS, Tovey DG, et al. Treatment of intestinal microsporidiosis with albendazole in patients with AIDS. *AIDS*. 1992;6:311–313.
374. Leder K, Ryan N, Spelman D, et al. Microsporidial disease in HIV-infected patients: a report of 42 patients and review of the literature. *Scand J Infect Dis*. 1998;30:331–338.
375. Molina JM, Goguel J, Sarfati C, et al. Potential efficacy of fumagillin in intestinal microsporidiosis due to *Enterocytozoon bieneusi* in patients with HIV infection: results of a drug screening study. The French Microsporidiosis Study Group. *AIDS*. 1997;11:1603–1610.

376. Eeftinck Schattenkerk JK, van Gool T, van Ketel RJ, et al. Clinical significance of small-intestinal microsporidiosis in HIV-1-infected individuals. *Lancet*. 1991;337:895–898.
377. Anwar-Bruni DM, Hogan SE, Schwartz DA, et al. Atovaquone is effective treatment for the symptoms of gastrointestinal microsporidiosis in HIV-1-infected patients. *AIDS*. 1996;10:619–623.
378. Bicart-Sée A, Massip P, Linas MD, et al. Successful treatment with nitazoxanide of *Enterocytozoon bieneusi* microsporidiosis in a patient with AIDS. *Antimicrob Agents Chemother*. 2000;44:167–168.
379. Albrecht H, Sobottka I, Stellbrink HJ, et al. Does the choice of *Pneumocystis carinii* prophylaxis influence the prevalence of *Enterocytozoon bieneusi* microsporidiosis in AIDS patients? *AIDS*. 1995;9:302–303.
380. Sharpstone D, Rowbottom A, Francis N, et al. Thalidomide: a novel therapy for microsporidiosis. *Gastroenterology*. 1997;112:1823–1829.
381. Kano T, Fukui H. Studies on *Pleistophora* infection in eel, *Anguilla japonica*. I. Experimental induction of microsporidiosis and fumagillin efficacy. *Fish Pathol*. 1982;16:193–200.
382. Higgins MJ, Kent ML, Moran JD, et al. Efficacy of the fumagillin analog TNP-470 for Nucleospora salmonis and *Loma salmonae* infections in Chinook salmon *Oncorhynchus tshawytscha*. *Dis Aquat Organ*. 1998;34:45–49.
383. Audemard A, Le Bellec ML, Carluer L, et al. Fumagillin-induced aseptic meningoencephalitis in a kidney transplant recipient with microsporidiosis. *Transpl Infect Dis*. 2012;14:E147–E149.
384. Diesenhouse MC, Wilson LA, Corrent GF, et al. Treatment of microsporidial keratoconjunctivitis with topical fumagillin. *Am J Ophthalmol*. 1993;115:293–298.
385. Coyle C, Kent M, Tanowitz HB, et al. TNP-470 is an effective antimicrosporidial agent. *J Infect Dis*. 1998;177:515–518.
386. Didier PJ, Phillips JN, Kuebler DJ, et al. Antimicrosporidial activities of fumagillin, TNP-470, ovalicin, and ovalicin derivatives in vitro and in vivo. *Antimicrob Agents Chemother*. 2006;50:2146–2155.
387. Zhang H, Huang H, Cali A, et al. Investigations into microsporidian methionine aminopeptidase type 2: a therapeutic target for microsporidiosis. *Folia Parasitol (Praha)*. 2005;52:182–192.
388. Alvarado JJ, Nemkal A, Sauder JM, et al. Structure of a microsporidian methionine aminopeptidase type 2 complexed with fumagillin and TNP-470. *Mol Biochem Parasitol*. 2009;168:158–167.
389. Garvey MJ, Ambrose PG, Ulmer JL. Topical fumagillin in the treatment of microsporidial keratoconjunctivitis in AIDS. *Ann Pharmacother*. 1995;29:872–874.
390. Rosberger DF, Serdarevic ON, Erlandson RA, et al. Successful treatment of microsporidial keratoconjunctivitis with topical fumagillin in a patient with AIDS. *Cornea*. 1993;12:261–265.
391. Wilkins JH, Joshi N, Margolis TP, et al. Microsporidial keratoconjunctivitis treated successfully with a short course of fumagillin. *Eye*. 1994;8:703–704.
392. Lowder CY, McMahon JT, Meisler DM, et al. Microsporidial keratoconjunctivitis caused by *Septata intestinalis* in a patient with acquired immunodeficiency syndrome. *Am J Ophthalmol*. 1996;121:715–717.
393. Orenstein JM, Seedor J, Friedberg DN, et al. Microsporidian keratoconjunctivitis in patients with AIDS. *MMWR Morb Mortal Wkly Rep*. 1990;39:188–189.
394. Das S, Wallang BS, Sharma S, et al. The efficacy of corneal debridement in the treatment of microsporidial keratoconjunctivitis: a prospective randomized clinical trial. *Am J Ophthalmol*. 2014;157:1151–1155.
395. Thanathanee O, Athikulwongse R, Anutarapongpan O, et al. Clinical features, risk factors, and treatments of microsporidial epithelial keratitis. *Semin Ophthalmol*. 2016;31:266–270.