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Cryptosporidium Taxonomy: Recent Advances and Implications for Public Health

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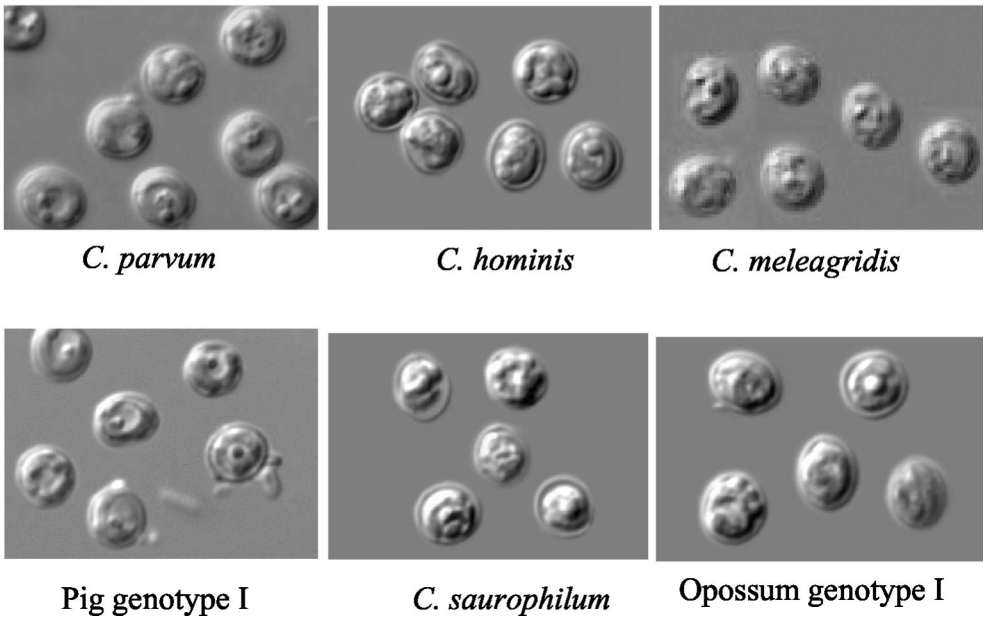

FIG. 1. Oocysts of *C. parvum* and some *C. parvum*-related species. Modified from reference 247.

INTRODUCTION

Cryptosporidium species are apicomplexan parasites that infect the microvillus border of the gastrointestinal epithelium of a wide range of vertebrate hosts, including humans. Infected individuals show a wide spectrum of clinical presentations, but the pathogenicity of *Cryptosporidium* varies with the species of parasites involved and the type, age, and immune status of the host. In many animals, *Cryptosporidium* infections are not associated with clinical signs or are associated with only acute, self-limiting illness. In some animals, such as reptiles infected with *Cryptosporidium serpentis* or individuals who are immunosuppressed, the infection is frequently chronic and can eventually be lethal.

Cryptosporidiosis is a frequent cause of diarrheal disease in humans, and several groups of humans are particularly susceptible to cryptosporidiosis. In developing countries, *Cryptosporidium* infections occur mostly in children younger than 5 years, with peak occurrence of infections and diarrhea in children younger than 2 years (21, 22, 154). Children can have multiple episodes of cryptosporidiosis, indicating that acquired immunity to *Cryptosporidium* infection is short-lived or incomplete (154, 248). In industrialized countries, epidemic cryptosporidiosis can occur in adults by the food-borne or waterborne route (117, 124, 191). In immunocompromised persons such as human immunodeficiency virus-positive (HIV^+) patients, the incidence and severity of cryptosporidiosis increases as the $CD4^+$ lymphocyte cell count falls, especially when it falls to below 200 cells/μl (152, 188, 198).

Because *Cryptosporidium* spp. infect humans and a wide variety of animals and because of the ubiquitous presence of *Cryptosporidium* oocysts in the environment, humans can acquire *Cryptosporidium* infections through several transmission routes (45, 80). In pediatric and elderly populations, especially in day care centers and nursing homes, person-to-person transmission probably plays a major role in the spread of *Cryptosporidium* infections (153, 214). In rural areas, zoonotic infections via direct contact with farm animals have been reported many times, but the relative importance of direct zoonotic transmission of cryptosporidiosis is not entirely clear (125). Numerous outbreaks of cryptosporidiosis due to contaminated food or water (drinking or recreational) have been reported in several industrialized nations, and studies have sometimes identified water as a major route of *Cryptosporidium* transmission in areas where the disease is endemic (67, 116, 156, 239). The sources and human infective potentials of *Cryptosporidium* oocysts in water, however, are largely unclear.

One major problem in understanding the transmission of *Cryptosporidium* infection is the lack of morphologic features that clearly differentiate one *Cryptosporidium* sp. from many others (60) (Fig. 1). Hence, one cannot be sure which *Cryptosporidium* sp. is involved when one examines oocysts in clinical specimens under a microscope. Another major problem is the inability to grow the organisms in large numbers from contaminated sources. Adding to the diagnosis problem and technical difficulties is the confusion in the taxonomy of *Cryptosporidium* spp., which is partially caused by the lack of consistency in the classification of protozoan parasites in general.

Associated with the problems in taxonomy and nomenclature is the public health importance of various *Cryptosporidium* spp. Without clear diagnostic features that allow the differentiation of *Cryptosporidium* spp. (Fig. 1; Table 1), we do not know the precise number of species infecting humans, the burden of disease (sporadic and outbreak related) attributable to different species or strains/genotypes, and the role of species and strains/genotypes in virulence or transmission in humans. These questions present challenges to our understanding of the epidemiology of cryptosporidiosis. Revision of *Cryptosporidium* taxonomy, therefore, is useful to our understanding of the biology, epidemiology and public health importance of various *Cryptosporidium* spp.

TABLE 1. Similarity in morphometric measurements of oocysts of *C. parvum* and *C. parvum*-related *Cryptosporidium* spp.[a]

Parasite	No. of oocysts measured	Length (μm)		Width (μm)		Ratio	
		Mean	95% CL[b]	Mean	95% CL	Mean	95% CL
C. parvum	44	4.85	0.05	4.39	0.04	1.11	0.02
C. hominis	44	4.91	0.07	4.28	0.06	1.15	0.02
C. meleagridis	55	4.93	0.06	4.40	0.05	1.12	0.02
Pig genotype I	56	5.05	0.07	4.41	0.08	1.15	0.02
C. saurophilum	20	4.94	0.13	4.49	0.14	1.14	0.03
Opossum genotype I	55	5.26	0.08	4.38	0.05	1.20	0.02

[a] Data are from reference 247.
[b] 95% CL, 95% confidence limits.

HISTORICAL PERSPECTIVE OF *CRYPTOSPORIDIUM* TAXONOMY

The first individual to establish the genus *Cryptosporidium* and to recognize its multispecies nature was Ernest Edward Tyzzer, who described the type species, *C. muris*, from the gastric glands of laboratory mice (225). He later published a more complete description of the life cycle (226) and subsequently described a second species, also from laboratory mice (227). *C. parvum* differed from the type species not only by infecting the small intestine rather than the stomach but also because the oocysts were smaller (227, 232).

Following the initial discovery of *Cryptosporidium*, over 50 years elapsed during which the parasite was commonly confused with other apicomplexan genera, especially members of the coccidian genus *Sarcocystis.* Because many *Sarcocystis* spp. have oocysts with thin walls that often rupture, releasing free sporocysts, and because each sporocyst contains four sporozoites like *Cryptosporidium* oocysts, a variety of named and unnamed species were erroneously assigned to the genus (11, 20, 53, 54, 77, 169, 224, 240). Subsequent ultrastructural studies, however, supported earlier light microscopy studies and reaffirmed endogenous stages of *Cryptosporidium* spp. to possess a unique attachment organelle (84, 93, 236). This attachment organelle, rather than the oocysts, is the key feature that currently defines the genus and family (230), but it has actually been an integral component of the taxonomic definition of the family since at least 1961 (102, 103, 105).

After the recognition of true differences between *Cryptosporidium* and *Sarcocystis*, the erroneous concept of strict host specificity (181) was applied to *Cryptosporidium* spp. This led to the creation of multiple new species including *C. agni* in sheep, *C. anserinum* in geese, *C. bovis* in calves, *C. cuniculus* in rabbits, *C. garnhami* in humans, and *C. rhesi* in monkeys (18, 23, 89, 104, 190). Subsequent cross-transmission studies demonstrated that *Cryptosporidium* isolates from different animals can frequently be transmitted from one host species to another, which ended the practice of naming species based on host origin and the synonymization of many of these new *Cryptosporidium* species as *C. parvum.* However, for a brief period, these very limited transmission studies were used as evidence for the monospecific nature of the genus *Cryptosporidium*, resulting in the widespread use of the name *C. parvum* for *Cryptosporidium* parasites from all kinds of mammals, including humans. Several *Cryptosporidium* parasites named during or before the period, such as *C. meleagridis* in turkeys (197), *C. wrairi* in guinea pigs (236), and *C. felis* in cats (90), however, survived because of the demonstrated biological differences from the established species *C. parvum* and *C. muris.* More recently, several other *Cryptosporidium* spp. were also named in a less haphazard fashion, such as *C. baileyi* in birds (49) and *C. saurophilum* in lizards (97), all based on biological differences from other established *Cryptosporidium* species.

In recent years, molecular characterizations of *Cryptosporidium* have helped to clarify the confusion in *Cryptosporidium* taxonomy and validate the existence of multiple species in each vertebrate class. As a result, several new species of *Cryptosporidium* have also been named. Thus, *C. andersoni* from cattle, *C. canis* from dogs, *C. hominis* from humans, and *C. molnari* from fish were all established by using multiple parameters that included not only morphology but also developmental biology, host specificity, histopathology, and/or molecular biology (4, 63, 111, 146).

SPECIES CONCEPT IN *CRYPTOSPORIDIUM*

One major reason for the long disputes in *Cryptosporidium* taxonomy is the difficulty in fulfilling the definition of biological species. The classical definition of species as groups of interbreeding natural populations reproductively isolated from other groups (119) is difficult to apply to many organisms like *Cryptosporidium*, because it is very difficult to conduct genetic crossing studies with many *Cryptosporidium* spp. Even though *Cryptosporidium* has a sexual stage and intraspecies sexual recombination has been demonstrated in *C. parvum* (65, 118), the huge reproductive potential of the parasite results in vast numbers of genetically similar parasites in localized areas. Therefore, mating in *Cryptosporidium* normally occurs between siblings. As a result, *Cryptosporidium* has a large bias toward a clonal population structure, as demonstrated by multilocus analysis (13, 72, 212).

Currently, morphology, especially oocyst measurements, represents the cornerstone of apicomplexan taxonomy. Measurements allow microscopists to identify large numbers of genera and morphologically distinct species, and the importance of a good morphologic description cannot be understated. Therefore, oocyst structure is usually one of the requirements for establishing a new species. However, for *Cryptosporidium*, morphology is not adequate by itself and should not be the sole criterion for naming a new species. Oocysts of many species are virtually identical in size, and similarities in oocyst structure have even caused confusion about the historical validity of several *Cryptosporidium* spp.

Because oocyst morphometrics alone is not entirely adequate for descriptions of new species of *Cryptosporidium*, other characteristics must be included in the taxonomic description. In many cases, experimental transmission followed by light microscopy and sometimes electron microscopy of endogenous stages has proven useful. Cases in point include the paper by Current and Reese (47), who provided an excellent account of the life cycle of *C. parvum* in experimentally infected mice by using a combination of light and electron microscopy. Current et al. (49) then published an account of the life cycle of *C. baileyi* in chickens and not only pointed out the morphologic differences in oocyst structure between *C. parvum* and *C. baileyi* but also showed that *C. baileyi* possessed a third type of merogonous stage not seen in *C. parvum.* More recently, Alvarez-Pellitero and Sitja-Bobadilla (4) utilized light microscopy and ultrastructure to provide an elaborate description of *C. molnari* in two species of marine teleosts. Nonetheless, a strict requirement for life cycle studies in all taxonomic works seems impractical for two reasons. First, distinct species may have similar endogenous development. Second, and equally important, many host species fail to lend themselves easily to animal experimentation. This latter point is especially true of oocysts derived from rare, exotic, venomous, excessively expensive, or very large hosts, making animal studies prohibitive.

Infectivity has sometimes been used to characterize and compare various *Cryptosporidium* isolates, and many carefully controlled infectivity studies have been published (162–164, 186, 215). Even though infectivity can be used as a good general indicator of host susceptibility and oocyst viability, real quantitative data are limited. Numerous variables affect parasite development, including dosage, oocyst age, oocyst storage conditions, the isolate employed, chemical pretreatments of the oocysts, the age, size, and previous exposure history of the host, whether mixed isolates are represented, and host genetics. For example, Upton and Gillock (233) showed how age and weight alone in ICR outbred suckling mice had dramatic impacts on the numbers of oocysts recovered from experimentally infected suckling mice. Enriquez and Sterling (59) examined *C. parvum* infections in 19 different strains of adult mice and found that the beige mouse (C57BL/6J-bgJ) harbored the highest levels of infection, with only scant numbers being found in other strains of mice.

In addition to infectivity, host specificity (the broad range of different hosts that can be infected by any one isolate) can prove highly useful when dealing with isolates derived from commonly encountered hosts. For example, one of the earliest ways in which *C. andersoni* in cattle was distinguished from the morphologically similar *C. muris* in rodents was by the fact that the former species was never infectious for outbred, inbred, neonatal, or immunocompetent mice (111). Likewise, *C. hominis* in humans has long been known to have a much narrower host range than the morphologically similar *C. parvum*, and cross-transmission studies help distinguish between the two (3, 146). Caution should be used when interpreting negative transmission results, however. Even though the lack of ability to infect mice and goats in cross-transmission studies was used as evidence for the separation of *C. andersoni* from *C. muris* (111), thus far it has been difficult to infect cattle of different ages and breeds with *C. andersoni* of bovine origin (9). The earlier conclusion that *C. hominis* does not infect experimental animals such as mice, calves, lambs, and pigs is apparently premature, since recent studies have clearly shown that calves, lambs, and piglets can be infected with *C. hominis* (3, 55). "Genotype switching" (a different genotype of oocysts obtained after inoculation with one genotype of oocysts) has also been observed in cross-transmission studies (63, 244). These results are important because they demonstrate that populations of oocysts derived from an individual animal may have low levels of contaminating minor species, which can further compound the interpretation of cross-transmission studies. Nonetheless, determination of at least some aspects of host range can provide highly useful information to support morphologic and genetic data and should be encouraged for as many species accounts as feasible.

Biochemical differences can potentially be used as one criterion in defining *Cryptosporidium* spp. Early on, restriction fragment length polymorphism analysis (RFLP) of genomic DNA (160), isozyme analysis (14–16, 56, 161), two-dimensional gel electrophoresis (122, 219), and protein or carbohydrate surface labeling of oocysts, sporozoites, or homogenates (112, 115, 155, 157, 158, 217, 218, 220, 222) were all used in an attempt to define both interspecific and intraspecific differences in *Cryptosporidium.* Differences in protein electrophoretic profiles between *C. parvum* bovine isolates and *C. wrairi* lent strong support to the validity of *C. wrairi* (221). Overall, these methods have proven expensive, technically challenging, and impractical. Not only is there no guarantee that different species would not have identical banding patterns when zymography or sodium dodecyl sulfate-polyacrylamide gel electrophoresis is used, but also relatively large numbers of parasites need to be used in some assays. Typically 10^7 to 10^8 highly purified oocysts are used, making repeatability, and sometimes even the initial experiments, impractical without passage and bioamplification in additional hosts.

Recent molecular studies have uncovered an overwhelming amount of genetic diversity within the genus *Cryptosporidium* (37, 50, 61, 141, 252, 255). In recent years, genetic differences have become a key essential element in defining several new *Cryptosporidium* spp., such as *C. andersoni*, *C. canis*, *C. hominis*, and *C. galli.* Thus far, genetic differences identified at the species or genotype level correlate well with other biological characteristics such as the spectrum of natural hosts and infectivity in cross-transmission studies. With further verification, genetic characteristics should play an even greater role in delineating and defining *Cryptosporidium* spp. Confusion exists, however, about how to distinguish interspecies differences from intraspecific allelic diversity and how much emphasis should be placed on results of molecular analysis.

Because of the uncertainty associated with the extent of intraspecific allelic variation in *Cryptosporidium* taxonomy, numerous *Cryptosporidium* genotypes have been described without a designation of species being given or with them all being lumped into *C. parvum.* Presently, the identification and naming of genotypes is based largely on host origin. When significant or consistent sequence differences from existing genetic data are identified, a new genotype is named after the host from which it was isolated. Although this genotype designation scheme generally reflects significant genetic differences among *Cryptosporidium* isolates and tends to correlate well with biological differences whenever data are available, not all geno-

TABLE 2. Valid *Cryptosporidium* species

Species	Major host	Minor host
C. muris	Rodents, bactrian camels	Humans, rock hyrax, moutain goats
C. andersoni	Cattle, bactrian camels	Sheep
C. parvum	Cattle, sheep, goats, humans	Deer, mice, pigs
C. hominis	Humans, monkeys	Dugongs, sheep
C. wrairi	Guinea pigs	
C. felis	Cats	Humans, cattle
C. canis	Dogs	Humans
C. meleagridis	Turkeys, humans	Parrots
C. baileyi	Chicken, turkeys	Cockatiels, quails, ostriches, ducks
C. galli	Finches, chicken, capercalles, grosbeaks	
C. serpentis	Snakes, lizards	
C. saurophilum	Lizards	Snakes
C. molnari	Fish	

types differ from each other to the same extent. Thus, some genotypes exhibit extensive nucleotide differences from congenerics whereas others are very similar to each other. The term "subgenotype" is sometimes used to describe relatively minor intragenotypic variations. The use of genotypes and subgenotypes tends to be difficult for researchers in other fields to comprehend. The application of a species designation for some of the well-characterized *Cryptosporidium* genotypes is useful since it helps relieve much of the confusion.

VALID *CRYPTOSPORIDIUM* SPECIES

Named species of *Cryptosporidium* that are currently considered valid species now include *C. andersoni* (cattle), *C. baileyi* (chicken and some other birds), *C. canis* (dogs), *C. felis* (cats), *C. galli* (birds), *C. hominis* (humans), *C. meleagridis* (birds and humans), *C. molnari* (fish), *C. muris* (rodents and some other mammals), *C. parvum* (ruminants and humans), *C. wrairi* (guinea pigs), *C. saurophilum* (lizards and snakes), and *C. serpentis* (snakes and lizards) (Table 2). Other morphologically distinct *Cryptosporidium* spp. have been found in fish (4), reptiles (234), birds (107), and mammals (61, 255) but have not been named.

Cryptosporidium Species of Mammals

Mammals represent the largest group of animals known to be infected with *Cryptosporidium* spp., probably due to the greater number of studies as a result of the perceived importance of these animals. The taxonomy of *Cryptosporidium* in mammals has been the subject of dispute since 1980, and for some time only two species (*C. parvum* as the intestinal species and *C. muris* as the gastric species) were recognized (229, 252). We now know that there is enormous biological and genetic diversity in mammalian *Cryptosporidium* spp., and because of a plethora of molecular studies, multiple new species have been discovered and described.

***Cryptosporidium muris* Tyzzer, 1907.** In 1907, Ernest Edward Tyzzer described a protozoan parasite that he frequently observed in the gastric glands of laboratory mice but not wild mice (225). The asexual meront stage contained six merozoites, each with a distinct nucleus. Sexual stages were observed and measured. All stages were thought to be extracellular, with an unusual knoblike attachment organelle similar to a gregarine epimerite. Spore (oocyst) formation was described, with oocysts measuring about 7 by 5 μm, and fecal-oral transmission was demonstrated. Although Tyzzer thought that the systematic position was uncertain, he nevertheless suggested the name *Cryptosporidium muris.* Three years later he extended the geographic range of the parasite in *Mus musculus* from North America to include England. A more detailed description of each life cycle stage (with measurements, drawings, and photographs) was later provided, and all stages were found to localize in the gastric glands of the stomach (226). Sporozoites liberated from oocysts in the gastric glands were thought to be autoinfectious; this has been found to be true for other *Cryptosporidium* spp. (47, 49). Nonetheless, pathology appeared to be slight. Experimental transmission to other mice was successful, but an attempt to infect a rat was not.

Experimental transmission studies using specific-pathogen-free laboratory rats revealed that a large type of *Cryptosporidium* oocysts from wild rats trapped in Osaka City developed only in the gastric glands of the stomach. The oocysts measured 8.4 by 6.3 μm and could be transmitted to uninfected rats. Oocysts from this study, identified as *C. muris* strain RN66, were later used for cross-transmission studies in which mice, guinea pigs, rabbits, dogs, and cats all became infected. Development occurred in the stomach and not the intestine, and oocysts were passed by all hosts (91). Based solely on morphology, *C. muris* or *C. muris*-like oocysts have been found in the feces of cattle in the United States (10, 232), Brazil (123, 182), Scotland (30), and Japan, (94) and in cattle and camels in Iran (159). Because species identification was not confirmed genetically or experimentally, many of these authors qualified their findings by calling the organism *C. muris*-like. Recent molecular characterizations of *C. muris* and *C. muris*-like parasites have indicated that all bovine isolates are *C. andersoni.* Recent studies have shown *C. muris* to be capable of infecting a wide range of additional hosts including hamsters, squirrels, Siberian chipmunks, wood mice (*Apodemus sylvaticus*), bank voles (*Clethrionomys glareolus*), *Dolichotis patagonum*, rock hyrax, bactrian camels, mountain goats, humans, and cynomolgus monkeys (10, 39, 52, 69, 82, 145, 168, 216, 223, 256; L. Xiao, unpublished data).

***Cryptosporidium andersoni* Lindsay, Upton, Owens, Morgan, Mead, and Blagburn, 2000.** *C. andersoni* infects the abomasum of cattle and produces oocysts morphologically similar to, but slightly smaller than, those of *C. muris* (111). It was named after Bruce Anderson, University of Idaho, the original finder of the parasite. Oocysts, passed fully sporulated, were ellipsoid, lacked sporocysts, and measured 7.4 by 5.5 (6.0 to 8.1 by 5.0 to 6.5) μm, with a length/width ratio of 1.35. Unlike those of *C. muris*, oocysts of *C. andersoni* were not infectious for outbred, inbred immunocompetent, or immunodeficient mice, nor were they infectious for chickens or goats. *C. andersoni* was recognized early on to be poorly infective not only to nonbovine hosts but also to cattle. Thus, oocysts derived from cattle, previously identified as *C. muris*-like, were not infectious for mice or even cattle (10). Similar oocysts from cattle were not transmissible to neonatal or adult BALB/c mice, SCID mice, common voles, bank voles, common field mice, desert gerbils,

guinea pigs, rats, rabbits, or goats; only Mongolian gerbils became infected (98). However, successful transmission of large *Cryptosporidium* oocysts from cattle to mice has been reported by Pavlásek (173) and Kaneta and Nakai (94). Whether these represent isolates of *C. andersoni*, an isolate of *C. muris* or *C. andersoni* with a wide host range, or contamination of the mice with *C. muris* is unknown. A Danish *C. andersoni* isolate was found to be infectious to cattle (58), and a so-called novel type of *C. andersoni* was identified in Japan based on its ability to infect SCID mice (196).

To overcome the conflicting cross-transmission data and the inability to morphologically differentiate oocysts of *C. andersoni* from those of *C. muris*, molecular methods have been employed in cross-transmission studies to confirm the species identification. Genetically confirmed *C. andersoni* infection has thus far been found only in cattle, bactrian camels, and a sheep (145, 256; U. M. Ryan, unpublished data).

***Cryptosporidium parvum* Tyzzer, 1912.** The most frequently reported species in mammals, *C. parvum*, was first found in mice (227). It was differentiated from *C. muris* based on its smaller oocyst size and its location only in the villi of the small intestine, most frequently near the tips. Transmission experiments from mouse to mouse always resulted in infection of the small intestine as opposed to the stomach. All life cycle stages were described, measurements were provided, and photographs and camera lucida drawings were included. Tyzzer remarked that stages were not strictly extracellular, but he did not consider them intracytoplasmic because they were in contact with the inner or cytoplasmic surface of the cell. Mature oocysts were ovoidal or spheroidal and did not exceed 4.5 μm in greatest diameter. Upton and Current (232) gave measurements of 5.0 by 4.5 (4.5 to 5.4 by 4.2 to 5.0) μm and a length/width ratio of 1.16 for viable oocysts, and Tilley et al. (222) reported that the oocysts measured 5.2 by 4.6 (4.8 to 5.6 by 4.2 by 4.8) μm with a length/width ratio of 1.15 (1.04 to 1.22). Tyzzer (227) also observed similar organisms in the small intestine of a rabbit. Frequently, *C. parvum* infection involves both the small intestine and the colon (228).

Over 150 species of mammals have been identified as hosts of *C. parvum* or *C. parvum*-like parasites. Most descriptions, however, have been based solely on microscopy, with no careful morphometric measurements or genetic or other biological data. Recent molecular characterizations, however, have shown that there is extensive host adaptation in *Cryptosporidium* evolution, and many mammals or groups of mammals have host-adapted *Cryptosporidium* genotypes, which differ from each other in both DNA sequences and infectivity. Thus, these genotypes are clearly being delineated as distinct species and include *C. hominis* (previously termed the human genotype or genotype 1), *C. parvum* (also termed the bovine genotype or genotype 2), and *C. canis* (the dog genotype). Other genotypes have been associated with mouse, pig, bear, deer, marsupial, monkey, muskrat, skunk, cattle, and ferret (255). Most of these organisms probably represent individual *Cryptosporidium* species.

It is possible that the *C. parvum* isolate originally found in laboratory mice by Tyzzer (227) might be what we now recognize as the *Cryptosporidium* mouse genotype (132, 134, 136, 138, 257). This is because Tyzzer (225) was able to easily infect adult mice whereas *C. parvum* senso stricto tends to produce very low-level infections in these hosts. However, because *C. parvum* is also occasionally found in mice and because no type specimens were originally deposited, we can never be sure what Tyzzer was actually working with. Therefore, when Upton and Current (232) provided a modern morphologic description of the oocysts and Current and Reese (47) provided in-depth life cycle and cross-transmission studies between mice and cattle, they essentially validated the name *C. parvum* for the bovine genotype. Even if one were to reject this argument and attempt to resurrect *C. bovis* Barker and Carbonell, 1974, for the bovine genotype in cattle (18), Article 23.9 of the *International Code of Zoological Nomenclature* (ICZN) specifically addresses reversal of priority in cases that may result in confusion. Specifically, Article 23.9.1.2 states that prevailing usage must be maintained when "the junior synonym or homonym has been used for a particular taxon, as its presumed valid name, in at least 25 works, published by at least 10 authors in the immediately preceding 50 years and encompassing a span of not less than 10 years." Clearly, *C. parvum* would fall into this category. We recommend the use of *C. parvum* for the *Cryptosporidium* parasites previously known as the bovine genotype and avoid the use of *C. parvum* broadly for *Cryptosporidium* in mammals.

Thus far, *C. parvum* is known to infect mainly ruminants (cattle, sheep, goats, and deer) and humans. Earlier reports of naturally occurring *C. parvum* infections in pigs and mice (129, 138) have yet to be confirmed by other researchers.

***Cryptosporidium canis* Fayer, Trout, Xiao, Morgan, Lal, and Dubey, 2001.** *Cryptosporidium* oocysts have been observed in the feces of dogs worldwide (reviewed in reference 63). Oocysts from the feces of a naturally infected dog measured 4.95 by 4.71 μm and had a length/width ratio of 1.05 (63). These oocysts were morphologically indistinguishable from those of *C. parvum* and possessed common surface antigens. Oocysts from the dog were infectious for a calf, but, unlike those of *C. parvum*, they were not infectious for neonatal BALB/c or for dexamethasone-treated and untreated C57BL6/N mice. Oocysts obtained from a human source were also infectious for a calf, and sequence analysis of the small-subunit (SSU) rRNA and HSP70 showed that these two isolates were identical to the dog genotype previously identified (144, 213, 257). Based on its ability to infect humans and bovines but its inability to infect mice, as well as significant genetic differences from other *Cryptosporidium* spp., the parasite was named *C. canis* (63). Confirmed *C. canis* infections have been found in dogs, coyotes, foxes, and humans (144, 177, 187, 248, 255, 257).

***Cryptosporidium felis* Iseki, 1979.** The first report of *Cryptosporidium* in cats included a description of the oocyst from the feces, basic observations of endogenous development, and some work on host specificity, and pathogenicity (90). Oocysts measuring 5 by 4.5 μm were fed to four cats, three 7-week-old ICR mice, and three 180- to 200-g guinea pigs. Oocysts were found only in the feces of three cats. Prepatent and patent periods were 5 to 6 and 7 to 10 days postinoculation, respectively. Mtambo et al. (148) obtained oocysts of two sizes from a farm cat and fed these to two lambs, which also shed similar sized oocysts, of 6.0 by 5.0 and 5.0 by 4.5 μm. Oocysts from the lambs were subsequently fed to 20 mice, of which 19 became infected, in contrast to 0 of 10 mice fed oocysts from the cat. Mtambo et al. (148) attributed the lack of infectivity for mice

of oocysts from the cat as possibly due to prolonged storage. However, another explanation may be that the lambs acquired an extraneous infection with *C. parvum* during the time they were being examined for oocyst shedding. Molecular testing would have been necessary to clarify these findings.

Even though the validity of *C. felis* was in doubt for some years, recent molecular characterizations at the SSU rRNA, ITS-1, HSP70, COWP, and actin loci support the concept of *C. felis* as a valid species. All *Cryptosporidium* isolates from cats characterized have thus far shown significant sequence differences from other known *Cryptosporidum* spp. and genotypes. In addition, all are very similar to each other even though they are from different geographic regions (134, 137, 211, 213, 242, 250). Confirmed *C. felis* infections have been found in cats, humans, and cattle (27, 33, 127, 137, 177, 187, 248, 257).

***Cryptosporidium wrairi* Vetterling, Jervis, Merrill, and Sprinz, 1971.** *Cryptosporidium wrairi* from the guinea pig (*Cavia porcellus*) was named as an acronym for the Walter Reed Army Institute of Research (236). Only small guinea pigs (weighing 200 to 300 g) were usually found to be infected. Infection was not associated with diarrhea or overt signs of coccidiosis, but only with enteritis (93, 236). Asexual and sexual stages were described, and fresh mucosal scrapings containing these stages were photographed. The times at which developmental stages were observed were recorded, but oocysts were never recognized as such. However, they may have been mistakenly misidentified as second-generation meronts containing four merozoites. Mucosa scraped from the distal ileum was delivered by gastric gavage to 3-week-old rabbits, chickens, and turkeys as well as young guinea pigs. Only the guinea pigs became infected, and this occurred only when scrapings were obtained 6 to 9 days after inoculation. The ultrastructure of all intracellular stages except mature microgametes, zygotes, and oocysts was described (237). Again, a micrograph identified as a second-generation meront containing three or four merozoites might actually be a developing oocyst.

Initially, cross-transmission studies suggested that *C. parvum* and *C. wrairi* might actually be the same species. Angus et al. (12) were able to transmit the parasite not only between guinea pigs but also to infant mice and lambs, even though it was not clear that this was the same species as that described by Vetterling et al. (236). Chrisp et al. (44) raised 23 monoclonal antibodies to *C. parvum* and 12 to *C. wrairi*, and they all reacted with equal intensity with the heterologous species. However, despite this close antigenic relationship, *C. wrairi* was not infectious for SCID mice whereas *C. parvum* was.

When *Cryptosporidium* from guinea pigs and *C. parvum* were compared morphologically, oocysts from guinea pigs measured 5.4 by 4.6 (4.8 to 5.6 by 4.0 to 5.0) μm and had a length/width index of 1.17 and those of *C. parvum* were similar in size and measured 5.2 by 4.6 μm with an index of 1.16 (221). All suckling mice inoculated with oocysts of *C. parvum* became infected, whereas most, but not all, mice inoculated with the guinea pig isolate became infected. However, mice inoculated with oocysts from guinea pigs produced on average 100-fold fewer oocysts than did mice infected with *C. parvum*, and the infections were sparse and patchy along the ileum. Electrophoretic profiles were similar, but ^{125}I surface labeling of outer oocyst wall proteins of *C. parvum* had a wide molecular size range of labeled bands whereas *Cryptosporidium* from guinea pigs had a banding pattern clustered between 39 and 66 kDa, with fewer bands greater than 100 kDa (221). Overall, these biological, immunological, and chemical labeling methods were confusing and inconclusive. More recently, molecular characterizations have identified significant differences between *C. parvum* and *C. wrairi* at multiple genetic loci (43, 147, 200, 201, 211, 213, 257). These combined data, along with the fact that naturally occurring *C. wrairi* infections have been found only in guinea pigs, strongly suggest that this organism is a different species from *C. parvum.*

***Cryptosporidium hominis* Morgan-Ryan, Fall, Ward, Hijjawi, Sulaiman, Fayer, Thompson, Olson, Lal, and Xiao, 2002.** *Cryptosporidium* parasites infecting humans, previously designated *C. parvum* human genotype, genotype 1, or genotype H, have been delineated as a separate species, *C. hominis*, based on molecular and biological differences (146). Numerous studies during the past several years showed not only a plethora of genetic and biological differences but also largely a lack of genetic exchange between this parasite and *C. parvum* (bovine genotype or genotype 2). *C. hominis* is morphologically identical to *C. parvum*, 4.6 to 5.4 by 3.8 to 4.7 μm (mean, 4.2 μm) with a length/width ratio of 1.21 to 1.15 (mean, 1.19). Unlike *C. parvum*, *C. hominis* is traditionally considered noninfective for mice, rats, cats, dogs, and cattle (73, 146, 185, 241, 242). However, more recently, *C. hominis* has been reported from a dugong and a lamb, and calves, lambs, and piglets can also be infected experimentally with at least some *C. hominis* isolates at high doses (3, 55, 73, 142). Pathogenicity studies with gnotobiotic pigs have shown the prepatent period to be longer than for *C. parvum* (8.8 and 5.4 days, respectively) and have also shown differences in parasite-associated lesion distribution and intensity of infection (146, 186). *C. hominis* and *C. parvum* also have different biological activities in cell culture (85). However, the number of isolates studied in animal and culture models has been small.

There appear to be distinct differences in oocyst shedding patterns between *C. hominis* and *C. parvum* in humans. A study in the United Kingdom revealed that *C. hominis* was detected in a significantly greater proportion of samples with larger numbers of oocysts whereas *C. parvum* was detected in a significantly greater proportion of the samples with small numbers of oocysts (121). Another study in Lima, Peru, reported that the duration of oocyst shedding in stool was significantly longer and the intensity of infections was significantly higher during *C. hominis* infections (248). There are also distinct geographical and temporal variations in the distribution of *C. parvum* and *C. hominis* infections in humans. In patients in the United Kingdom, *C. parvum* was more common during spring whereas *C. hominis* was more common in late summer and autumn in those with a history of foreign travel (120).

Genetic characterization of *C. hominis* and *C. parvum* has consistently demonstrated distinct differences between the two species at a wide range of loci (5–7, 15, 16, 25, 26, 31, 32, 35, 37, 68, 72, 76, 82, 83, 120, 121, 127, 130–134, 136, 161, 166, 168, 179, 183, 185, 194, 199–201, 206, 209–213, 235, 241–244, 248, 250, 254, 256, 257). There are also fundamental differences in ribosomal gene expression between *C. hominis* and *C. parvum*, since the latter constitutively expresses two types of rRNA genes (type A and type B [101]) whereas more than two transcripts have been detected in *C. hominis* (251). In addition,

despite the large number of isolates examined at multiple unlinked loci from a wide range of geographical locations, putative recombinants between *C. hominis* and *C. parvum* have never been explicitly identified (118). Although some interspecific recombination has suggested by several research groups (65, 100, 205, 243), the significance or extent of any recombination is not yet fully clear. If recombination between species does occur, it seems to be very limited.

The *Cryptosporidium* monkey genotype, which appears to be a variant of *C. hominis*, has been found in rhesus monkeys (255, 257).

Cryptic species. When Tyzzer initially described *C. parvum* in the small intestine of mice in 1912, he was working with adult mice (227). Recent research, however, has revealed that mice harbor a genetically distinct form of *Cryptosporidium*, the mouse genotype, which is different from what we commonly know as *C. parvum* (this latter species was previously referred to as the *C. parvum* bovine genotype). Only rarely is *C. parvum* (bovine genotype) found naturally in mice since it is predominantly a parasite of ruminants and some humans (136, 138, 257). Therefore, as explained above, it is likely that the species described by Tyzzer in 1912 was not *C. parvum* (bovine genotype) but in fact the mouse genotype. Because the bovine genotype of *Cryptosporidium* retains the name *C. parvum* and because the mouse genotype is biologically and genetically distinct, the mouse genotype will almost certainly be named as a new species shortly.

There are probably many other cryptic *Cryptosporidium* species in mammals, all of which were previously assumed to be *C. parvum.* Thus far, nearly 20 *Cryptosporidium* genotypes with uncertain species status have been collectively found in pigs (two genotypes), sheep, horses, cattle, rabbits, marsupials, opossums (two genotypes), ferrets, foxes, deer (two genotypes), muskrats (two genotypes), squirrels, bear, and deer mice (255). The genetic distances among these *Cryptosporidium* parasites are greater than or comparable to those among established intestinal *Cryptosporidium* species. Limited cross-transmission studies have shown biological differences among some of the genotypes (57), some of which have even shown oocyst morphology different from that of *C. parvum* (Table 1; Fig. 1).

Cryptosporidium Species of Birds

Although infections have been found in over 30 species of birds (62, 107, 160, 203), only three avian *Cryptosporidium* spp. have been named: *C. meleagridis*, *C. baileyi*, and *C. galli.* These three *Cryptosporidium* spp. can each infect a broad range of birds, but they differ in predilection sites. Even though both *C. meleagridis* and *C. baileyi* are found in the small and large intestine and bursa, they differ significantly in oocyst size and only *C. baileyi* is also found in the respiratory tissues such as the conjunctiva, sinus, and trachea. In contrast, *C. galli* infects only the proventriculus.

***Cryptosporidium meleagridis* Slavin, 1955.** Developmental stages of a parasite that conformed to those found by Tyzzer (225) from the small intestine of mice were found on the villus epithelium in the terminal one-third of the small intestine of turkeys in Scotland (197). Based on dried smears stained by the MacNeal-modified Romanowsky method, the cytology and measurements of merozoites, trophozoites, meronts, gametes, and oocysts were ascertained and the parasite was named *C. meleagridis.* Oval oocysts measuring 4.5 by 4.0 μm (197) appeared indistinguishable from those of *C. parvum.* Lindsay et al. (110) gave measurements of 5.2 by 4.6 (4.5 to 6.0 by 4.2 to 5.3) μm for viable oocysts from turkey feces. Although enormous numbers were observed in smears, sporozoites could not be identified within them (197). Illness with diarrhea and a low death rate in 10- to 14-day-old turkey poults was associated with the parasite, which completed its life cycle on the villus epithelium without appearing to invade host tissues (197). No attempts were made at this time to transmit this parasite to other hosts, although subsequent studies have demonstrated that turkeys and chickens are susceptible to infection after oral inoculation with *C. meleagridis* oocysts (107).

Subsequent molecular analysis of a turkey isolate in North Carolina and a parrot isolate in Australia at the SSU rRNA, HSP70, COWP, and actin loci demonstrated the genetic uniqueness of *C. meleagridis* (143, 211, 213, 250, 257). Viable oocysts measured 5.1 by 4.5 μm (143). When the morphology, host specificity, and organ location of *C. meleagridis* from a turkey in Hungary were compared with those of a *C. parvum* isolate, phenotypic differences were small but statistically significant (202). Oocysts of *C. meleagridis* were successfully transmitted from turkeys to immunosuppressed mice and from mice to chickens. Sequence data for the SSU rRNA gene of *C. meleagridis* isolated from turkeys in Hungary were found to be identical to the sequence of a *C. meleagridis* isolate from North Carolina. Even though it has been suggested *C. meleagridis* may be *C. parvum* (40, 203), results of molecular and biological studies have confirmed that *C. meleagridis* is a distinct species (135, 143, 202, 211, 213, 250, 255, 257).

C. meleagridis is apparently a misnomer since it infects other avian hosts (for example, parrots), not just turkeys (135, 143). It is also the third most common *Cryptosporidium* parasite in humans (178, 248). Several subtypes of *C. meleagridis* have been described based on multilocus analysis (75).

***Cryptosporidium baileyi* Current, Upton, and Haynes, 1986.** A second species of avian *Cryptosporidium*, originally isolated from commercial broiler chickens, was named *C. baileyi* based on its life cycle and morphologic features (49). The species was named in honor of the late W. S. Bailey, then President of Auburn University, for his pioneering work on the biology of *Spirocerca lupi.* The prepatent period was 3 days, and the patent period lasted 20 and 10 days for birds inoculated at 2 days of age and at 1 or 6 months of age, respectively. Developmental stages, found in the microvillus region of enterocytes of the ileum and large intestine, were described, measured, and photographed, and the time of their first appearance was noted. Heavy infection of the bursa of Fabricius (BF) and cloaca did not result in clinical illness. Thin-walled oocysts were observed, but most were thick walled. Viable oocysts measured 6.2 by 4.6 (5.6 to 6.3 by 4.5 to 4.8) μm and thus were much larger and more elongate than those of *C. meleagridis.* Mice, goats, and quail inoculated with oocysts did not become infected, but limited life cycle stages were observed in some turkey poults, and heavy infections developed only in the BF in 1-day-old ducks and 2-day-old geese (49). Sporozoites excysted in vitro and inoculated intranasally produced upper respiratory infections similar to those reported for naturally infected broil-

ers (49). *C. baileyi* was found in the colon, cloaca, and BF as well as at several respiratory sites in black-headed gulls (*Larus ridibundus*) younger 30 days in the Czech Republic (172). Oocysts measuring 6.4 by 4.9 μm were successfully transmitted from gulls to 4-day-old chickens (*Gallus gallus domestica*).

C. baileyi is probably the most common avian *Cryptosporidium* sp. and has so far been found in chicken, turkeys, ducks, cockatiels, a brown quail, and an ostrich (108, 135; L. Xiao and U. M. Ryan, unpublished data). Hence, a wide range of birds can be infected with *C. baileyi.* High morbidity and mortality are often associated with *C. baileyi* respiratory infections of birds, especially broiler chickens (107).

***Cryptosporidium galli* Pavlasek, 1999.** A third species of avian *Cryptosporidium* was first found by Pavlásek (174) in hens (*Gallus gallus domesticus*) on the basis of biological differences. The parasite has recently been redescribed on the basis of both molecular and biological differences (195). *C. galli* appears to be associated with clinical disease and high mortality (135, 174, 175, 195). Oocysts are larger than those of other avian species of *Cryptosporidium* and measure 8.25 by 6.3 (8.0 to 8.5 by 6.2 by 6.4) μm, with a length to width ratio of 1.3 (175). Oocysts were infectious for 9-day-old but not 40-day-old chickens (185). Unlike other avian species, life cycle stages of *C. galli* developed in epithelial cells of the proventriculus and not the respiratory tract or small and large intestines (174). DNA sequence analysis of three different loci confirmed that *C. galli* was distinctively different from *C. baileyi* and *C. meleagridis* and was related to the gastric *Cryptosporidium* parasites found in reptiles and mammals (*C. serpentis*, *C. muris*, and *C. andersoni*).

Blagburn et al. (24) may also have detected *C. galli* in birds when they used light and electron microscopy to characterize *Cryptosporidium* parasites in the proventriculus of an Australian diamond firetail finch that died of acute diarrhea. A subsequent publication also identified a species of *Cryptosporidium* infecting the proventriculus in finches and inadvertently proposed the name *C. blagburni* in Table 1 of the paper (135). However, Pavlásek (174, 175) had provided a detailed description of what appeared to be the same parasite and named it *C. galli.* More recent molecular analyses have revealed *C. galli* and *C. blagburni* to be the same species (195).

Confirmed hosts of *C. galli* include finches (Spermestidae and Fringillidae), domestic chickens (*G. gallus*), capercaille (*Tetrao urogallus*), and pine grosbeaks (*Pinicola enucleator*) (195). Morphologically similar oocysts have been observed in a variety of exotic and wild birds including members of the Phasianidae, Passeriformes, and Icteridae (195). Future studies are required to determine the extent of the host range for *C. galli.* Genetic heterogeneity probably exists in *C. galli*, since one finch isolate had significant sequence divergence in the SSU rRNA gene from the other *C. galli* isolates from finches and other birds (195).

Cryptic species. Based on limited biological and molecular studies, it appears that several other avian *Cryptosporidium* spp. are distinct species as well (66, 109, 135, 255). It was suggested that bobwhite quails may harbor a different *Cryptosporidium* species, which had oocysts similar to those of *C. meleagridis* but differed from *C. meleagridis* by infecting the entire small intestine and by causing severe morbidity and mortality (81, 86, 193). Another species is possibly present in ostriches, since this parasite has oocysts similar to *C. meleagridis* but is not infective to freshly hatched chickens, turkeys, or quail (66). These observations have yet to be confirmed by molecular characterizations. More recently, a new genotype of *Cryptosporidium* parasites has been found in an ostrich, but it is related to *C. baileyi* rather than *C. meleagridis* (U. M. Ryan and L. Xiao, unpublished data). Several other new *Cryptosporidium* spp. have been found in birds by molecular analysis, such as a duck genotype in a black duck and two goose genotypes in Canada geese, all of which are related to intestinal *Cryptosporidium* species (135, 255). Another new genotype has recently been found in a Eurasian woodcock. Even though it clustered with *C. galli* in phylogenetic analysis, it may represent a separate species (Ryan and Xiao, unpublished).

Cryptosporidium Species of Reptiles

Of all the animals, reptiles, especially snakes, are affected most severely by cryptosporidiosis due to the chronic and detrimental nature of the infection in reptiles. Even though a high prevalence of *Cryptosporidium* infections has sometimes been found in captive reptiles, few studies have attempted to identify the species structure of the parasite in reptiles. For quite some time, one species, *C. serpentis*, was the only species identified in reptiles. More recently, however, *C. saurophilum* was described in lizards (97).

***Cryptosporidium serpentis* Levine, 1980.** *C. serpentis* was named by Levine (104) based solely on a clinical report by Brownstein et al. (29) and using the rationale that species of *Cryptosporidium* had been customarily differentiated by association with their hosts. Based strictly on the ICZN, *C. serpentis* remained a nomen nudum until it was validated by morphologic and other biological data in a study by Tilley et al. (222). These authors reported oocysts to measure 6.2 by 5.3 (5.6 to 6.6 by 4.8 to 5.6) μm, with a length/width ratio of 1.16 (1.04 to 1.33). Another group (Xiao, unpublished) obtained measurements of 5.9 by 5.1 μm and a length/width ratio of 1.17. Levine (104) noted that cross-transmission among snakes had not been attempted and that therefore the name *C. serpentis* might encompass more than one species. Indeed, Brownstein et al. (29) reported that 14 snakes of three genera and four species (*Elaphe guttata*, *Elaphe subocularis*, *Crotalus horridus*, and *Sansinia madagascarensis*) in two zoological parks over a period of 7 years had severe chronic hypertrophic gastritis. Signs included postprandial regurgitation and firm midbody swelling. Gross and histological pathology were described. All developmental forms of *Cryptosporidium* were identified by ultrastructure in the gastric mucosa. Unlike avian and mammalian cryptosporidiosis, infections occur in mature snakes, the clinical course is usually protracted, and once infected most snakes remained infected (29). Between 1986 and 1988, 528 reptiles from three continents were examined for *Cryptosporidium* and 14 specimens representing eight genera and 11 species were found infected (234). Although in most cases the investigators were unable to examine the hosts for the site of infection, they presented a morphologic and statistical study of the oocysts of nine isolates and concluded that these isolates could be placed in five separate groups. Without additional isolates to determine the sites of infection and life cycles, the authors were reluctant to name new species.

Thus far, most isolates from snakes characterized by molecular analysis appear to be related to other gastric *Cryptosporidium* spp. (*C. muris*, *C. andersoni*, and *C. galli*) found in mammals and birds (139, 211, 213, 250, 255, 256). More recently, *C. serpentis* was found in 25 of 44 isolates from various species of snakes, some of which showed the same clinical signs and pathologic changes described by Brownstein et al. (29). It was also found in gastric washings from three snakes with clinical cryptosporidiosis; based on the collective data, *C. serpentis* seems to be the most common *Cryptosporidium* sp. (Xiao, unpublished).

Despite the name, *C. serpentis* apparently also infects lizards. Two isolates from savannah monitors were shown to be genetically related to *C. serpentis* based on sequence analysis of the SSU rRNA gene (256). More recently, PCR-RFLP and sequence analysis of the SSU rRNA gene of 24 isolates from various lizards identified 10 *C. serpentis* infections, and data suggest that *C. serpentis* is at least as common as *C. saurophilum* in lizards (Xiao et al., unpublished). There may be some host adaptation, since there are some minor differences between snake and lizard isolates in the sequence of the SSU rRNA and actin genes (211, 256). It is possible, however, to cross-transmit these two *C. serpentis* subtypes between snakes and lizards (Xiao, unpublished).

***Cryptosporidium saurophilum* Koudela and Modry, 1998.** *C. saurophilum* was named following an extensive study of the feces or intestinal contents from 220 wild and captive lizards of 67 species (97). Six species of lizards in five genera were found to be passing oocysts, and Schneider's skink (*Eumeces schneideri*) was designated the type host. The site of infection in the type host was the intestine and cloaca. Oocysts measured 5.0 by 4.7 (4.4 to 5.6 by 4.2 to 5.2) μm, with a length/width ratio of 1.09. No pathological changes were found in the intestine and cloaca of adult lizards, but weight loss, abdominal swelling, and mortality occurred in some colonies of juvenile geckos (*Eublepharis macularius*). Oocysts from *E. schneideri*, *Varanus prasinus*, and *Mabuya perrotetii* were inoculated into five genera of lizards, and all the animals became infected. Snakes, chickens, BALB/c mice, and SCID mice were also inoculated but did not become infected. Based on oocyst size, site of infection, and cross-transmission studies, the name *C. saurophilum* was proposed for this species of *Cryptosporidium* in lizards.

Molecular characterizations support the existence of *C. saurophilum.* A *Cryptosporidium* isolate from a desert monitor (*Varanus griseus*) was shown to be genetically distinct from *C. serpentis* and to be more closely related to the intestinal *Cryptosporidium* spp. of mammals and birds (257). More recently, this parasite was found in 9 of 24 *Cryptosporidium* isolates from monitors, iguanas, and geckoes (Xiao, unpublished). Because of oocyst morphology (4.94 by 4.49 μm, with a length/width ratio of 1.14 [Table 1]), it was concluded that these lizard parasites were *C. saurophilum.*

Even though *C. saurophilum* was originally described as a lizard parasite, it has been found recently in two captive snakes in Missouri. Both snakes were heavily infected, and one had clinical signs of diseases (Xiao, unpublished). A group of six snakes housed together with four lizards in the same room in Maryland also had *C. saurophilum* infections, but with much lower intensity than the infection of the four lizards (Xiao, unpublished). Two corn snakes and two leopard geckos were inoculated with *C. saurophilum* oocysts isolated from a bull snake. Only the geckos became infected; one had parasites in the intestine only, whereas the other had parasites in both the stomach and intestine (Xiao, unpublished). Therefore, it seems that snakes can also be infected with *C. saurophilum* and that *C. saurophilum* infection in snakes is not totally restricted to the intestine. Previously, some snakes were found infected with a *Cryptosporidium* sp. restricted to the intestine (28).

Cryptic species. More *Cryptosporidium* species are likely to be present in reptiles, because an earlier study identified at least five morphotypes in wild and captive reptiles (234). Some of these morphotypes might also represent oocysts of *C. muris* and the *Cryptosporidium* mouse genotype or other species from ingested prey (pseudoparasites) with cryptosporidiosis. These *Cryptosporidium* spp. are frequently found in snakes without clinical signs of infection (139, 234; Xiao, unpublished). How many of these represent pseudoparasites and how many represent pathogens is currently unknown. Turtles and tortoises are known to be infected with distinct gastric and intestinal forms of *Cryptosporidium* (78, 79, 255), and gekkonids harbor a distinct cloacal form (231). Several unknown intestinal *Cryptosporidium* genotypes have been identified in snakes and lizards by molecular analysis (255; Xiao, unpublished).

Another new species, *C. varanii* Pavlasek, Lavickova, Horak, Kral, and Kral, 1995, was found in an Emerald monitor (*Varanus prasinus*) living in the Czech Republic but captured in New Guinea (176). Oocysts of this parasite measured 4.8 by 4.7 (4.8 to 5.1 by 4.4 to 4.8) μm with a length/width ratio of 1.03. Parasite stages were found in the intestine, especially in the caudal section. It remains to be determined whether *C. varanii* is actually *C. saurophilum.*

Cryptosporidium Species of Fish

Descriptions of *Cryptosporidium*-like parasites in fish were reviewed by Alvarez-Pellitero and Sitja-Bobadilla (4). Two named species of *Cryptosporidium* have been found in fish, *C. nasoris* Hoover, Hoerr, Carlton, Hinsman, and Ferguson, 1981 (87), and *C. molnari* Alvarez-Pellitero and Sitja-Bobadilla, 2002 (4).

***Cryptosporidium molnari* Alvarez-Pellitero and Sitja-Bobadilla, 2002.** *Cryptosporidium* infection in gilthead sea bream and European sea bass from the Atlantic, Cantabric, and Mediterranean coasts of Spain was studied over a period of 3 years (4). Mucosal scrapings, feces, and fixed tissue from gilthead sea bream were examined by light microscopy, and infected stomachs were studied by electron microscopy. Fixed tissues from European sea bass were examined by light microscopy. Developmental stages were described in detail, with numerous photomicrographs and electron micrographs, and oocyst measurements were obtained from fresh and fixed specimens. Most parasite stages were located at the surface of epithelial cells in the stomach but were seldom found in the intestine. Similar to *Cryptosporidium* in other piscine species, zygotes and oocysts were located mainly in the basal portion of the epithelium. Oocysts, nearly spherical (length/width ratio, 1.05), had a great size range but averaged 4.72 by 4.47 μm. Pathological effects, mostly in fingerlings and juvenile fish, were seen in over 24% of gilthead sea bream versus 4.6% of sea bass. Histological damage was documented. The species was named in honor of the

Hungarian parasitologist Kalmar Molnar because of his extensive contribution to fish parasitology.

Unfortunately, no molecular characterization of *C. molnari* has been conducted thus far, which may cause problems for the identification and naming of other *Cryptosporidium* spp. in fish. Based on the predilection site, *C. molnari* may be genetically related to other gastric *Cryptosporidium* spp. such as *C. muris*, *C. andersoni*, and *C. galli.* However, molecular characterization is needed to determine if this is indeed the case.

Cryptic species. *Cryptosporidium nasoris* (syn. *C. nasorum*) should be regarded as a nomen nudum. Only developmental stages of the parasites on the microvillous surface of intestinal epithelial cells were described by light and electron microscopy. No measurements of viable oocysts were provided, and no taxonomically useful diagnostic features were presented. The species was named solely on the basis of the presumed host specificity of *Cryptosporidium* spp.

Some parasites in fish have been identified only as *Cryptosporidium* sp. (34, 99, 149). Others have been placed in a genus, *Piscicryptosporidium*, primarily because oocysts are located deep within epithelial cells and retain residual microvilli (170). These authors described two new species, *P. reichenbachklinkei* from gourami and *P. cichlidaris* from cichlids, based solely on transmission electron microscopy. However, additional molecular studies need to be conducted to determine whether the *Cryptosporidium* spp. of fish warrant generic status, and we regard the parasites as species inquirenda. In any case, it is almost certain that fish harbor more than one species of *Cryptosporidium*, since *Cryptosporidium* spp. have also been found in the intestines of various fish (4).

HOST-PARASITE COEVOLUTION AND HOST ADAPTATION IN *CRYPTOSPORIDIUM*: IMPLICATIONS FOR TAXONOMY

As with other well-studied parasites, recent studies indicate that both host-parasite coevolution and host adaptation exist in the evolution of *Cryptosporidium.* Phylogenetic analyses of *Cryptosporidium* spp. at the SSU rRNA, HSP70, and actin loci all support a general genetic structure of the genus *Cryptosporidium*, with all gastric and intestinal *Cryptosporidium* spp. forming their own monophyletic groups. Within each group, parasites of reptiles form the basal branches and most mammalian parasites form later branches (Fig. 2). The placement of *C. baileyi* is still uncertain; in the SSU rRNA and actin-based phylogenetic trees, it groups with the intestinal parasites, whereas in the HSP70-based tree, it groups with the gastric parasites. The branch orders of individual species or genotypes within the gastric or intestinal species differ somewhat among the three genetic loci (255). These differences may be due to differences in the rate and nature of genetic variations among the three genes. The SSU rRNA gene of *Cryptosporidium* evolves slowly, with sequence variations limited to several regions of the gene. In contrast, HSP70 and actin are highly polymorphic over the entire length of the genes and the genetic differences between gastric and intestinal *Cryptosporidium* in the two genes approach those between *Cryptosporidium* and *Plasmodium.* Thus, the HSP70 and actin genes are probably very useful to infer the genetic relationship of closely related *Cryptosporidium* spp. because of the high sequence heterogeneity but less useful to assess the relationship between distant members because of homoplasy.

Host-Parasite Coevolution

Genetically related hosts often harbor related species of *Cryptosporidium.* This is supported by the following observations. (i) All three phylogenetic analyses support a close relatedness of *Cryptosporidium* parasites from North American and Australian marsupials. (ii) The *Cryptosporidium* species from coyotes and one of the two genotypes from foxes are related to *C. canis* from dogs. (iii) *C. canis* from dogs and the *Cryptosporidium* genotypes from coyotes and foxes form a cluster with the *Cryptosporidium* parasite from bears. (iv) The *Cryptosporidium* deer genotype appears to be related to a newly identified genotype (the bovine genotype B) in cattle. (v) *Cryptosporidium* parasites from primates (*C. hominis* and the monkey genotype), lagomorphs (rabbit genotype), and rodents (mouse genotype), which have been shown recently to be genetically related mammals, Euarchonotoglires (150, 151), form a monophyletic group. These observations collectively support a theory of host-parasite coevolution in the genus *Cryptosporidium* (255).

It is difficult to determine the time of emergence of the genus *Cryptosporidium* parasites because of the lack of fossil records and controversy in the use of a molecular clock to attempt to time evolutionary events. However, Carreno et al. (36) have shown that *Cryptosporidium* spp. form a sister clade with the gregarines, parasites of invertebrates, suggesting a very early emergence of the parasite from the rest of the Apicomplexa. However, the two-way split of gastric and intestinal *Cryptosporidium* spp. probably occurred before the emergence of fish and reptiles, since both types of parasites occur in fish and reptiles. In addition, reptilian *Cryptosporidium* spp. form a basal position for both groups in all phylogenetic analyses, and the extent of sequence differences between the gastric and intestinal *Cryptosporidium* spp. is very large (>5.7% in the SSU rRNA gene). This is supported by the small genetic distance (0.38%) between *Cryptosporidium* species of the American (opossum genotype I) and Australian (marsupial genotype) marsupials, which have been separated from each other for over 50 million years because of continental drift. Further studies of parasites from amphibians and fish would be useful to the understanding of *Cryptosporidium* evolution.

Two exceptions to the hypothesis of host-parasite coevolution in the genus *Cryptosporidium* are *C. parvum* (the bovine genotype) and *C. meleagridis.* On the parasite side, *C. parvum* is genetically very similar to the species found in mice (the mouse genotype) and forms a monophyletic group with *C. hominis* and the monkey and rabbit genotypes (Fig. 2). On the host side, rodents, primates, and lagomorphs originated from a common ancestor different from the ancestor of ruminants (150, 151). It is possible that *C. parvum* was originally a parasite of rodents that has been recently established in cattle. This host expansion in *Cryptosporidium* is supported by the ability of *C. parvum* to infect several types of mammals, including rodents. The genetic diversity of *C. parvum* is lower than that of *C. hominis* (205), also supporting the theory of recent introduction of the parasites into ruminants. Cattle also apparently have their own *Cryptosporidium* genotype, the unnamed *Cryp-*

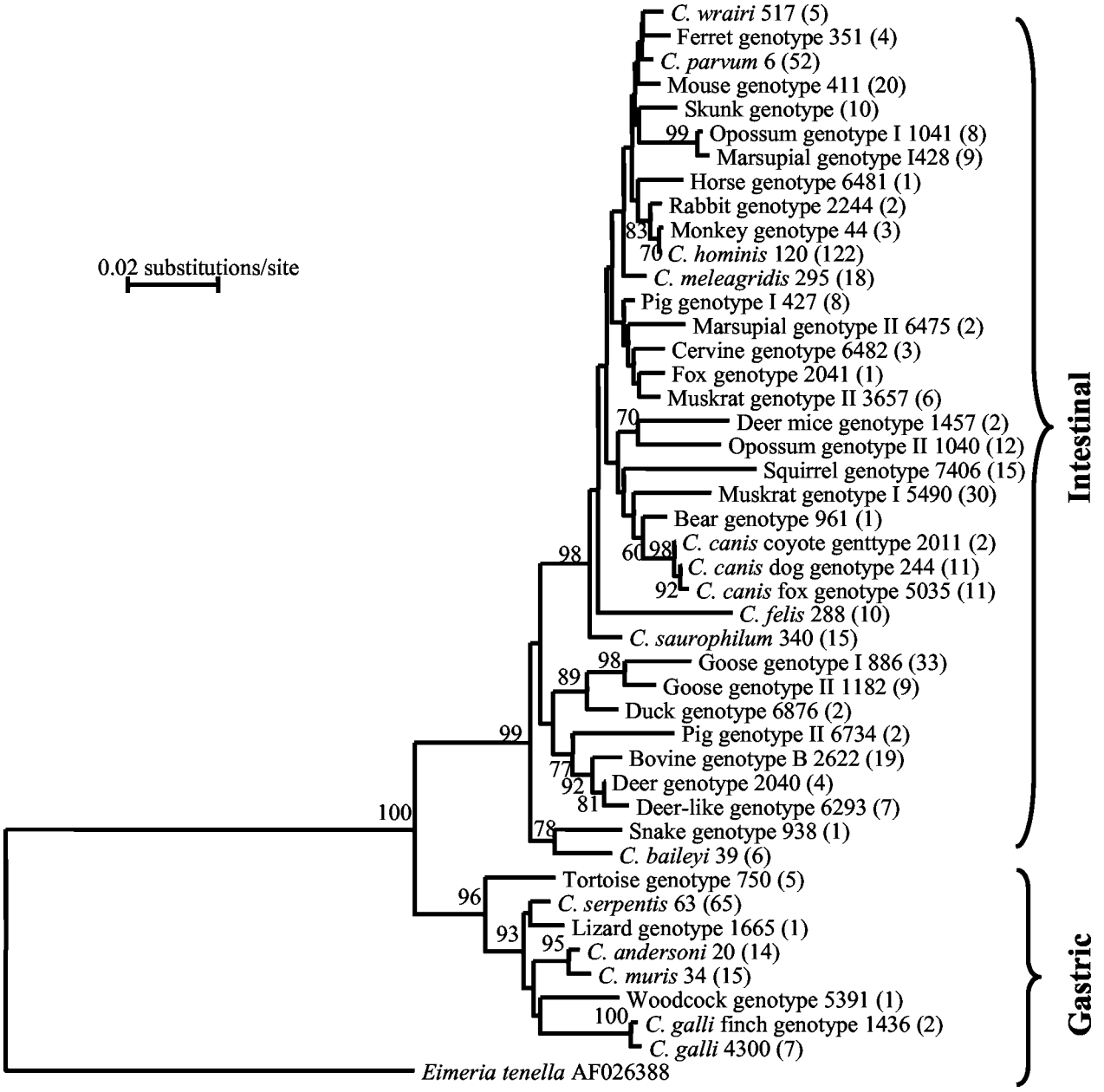

FIG. 2. Genetic relationship among named *Cryptosporidium* species and unnamed genotypes inferred by a neighbor-joining analysis of the partial SSU rRNA gene. Values on branches are percent bootstrapping using 1,000 replicates. Numbers following species or genotypes are isolate identifications used in the construction of the phylogenetic tree, whereas numbers in parentheses are the number of isolates sequenced. Modified from reference 255.

tosporidium bovine genotype B, which is genetically related to the *Cryptosporidium* deer genotype (255). This latter species infects a ruminant related to cattle. It is unclear why this *Cryptosporidium* genotype is not very prevalent in cattle. It is possible that immunity induced by *C. parvum* has limited the prevalence of the *Cryptosporidium* bovine genotype B in cattle. *C. parvum* predominantly infects neonatal ruminants, including calves, lambs, and kids, at or near the time of birth (62).

Host expansion has also apparently occurred in *C. meleagridis.* Unlike *C. baileyi* and *Cryptosporidium* spp. in ducks and geese, which have near-basal positions in the intestinal *Cryptosporidium* group in all phylogenetic analyses, *C. meleagridis* is always placed in a clade containing most mammalian *Cryptosporidium* spp. and is most closely related to *C. parvum*, *C. hominis*, *C. wrairi*, and monkey, mouse, and rabbit genotypes (Fig. 2), all of which are parasites of the Euarchontoglires (primates, lagomorphs, and rodents). Thus, *C. meleagridis* originally may have been a mammalian *Cryptosporidium* sp. that has been subsequently established in birds. This is supported by the recent findings of the ability of *C. meleagridis* to infect a wide range of mammals, including humans, rodents, gnotobiotic pigs, and calves (2, 178, 202, 248; L. Xiao and L. Ward,

unpublished data), which is certainly not the case with *C. baileyi.*

Host Adaptation

As expected, as the host evolves, *Cryptosporidium* spp. gradually become adapted to a particular group of animals and develop some host specificity. This is especially true for the intestinal *Cryptosporidium* spp. in mammals. In addition to the named species such as *C. parvum*, *C. hominis*, *C. wrairi*, *C. canis*, and *C. felis*, there are many *Cryptosporidium* genotypes with no established species names, such as the mouse, horse, sheep, ferret, cervine, muskrat, fox, skunk, deer, deer mouse, bear, and marsupial genotypes, pig genotypes I and II, opossum genotypes I and II, and bovine genotype B (Fig. 2). All of these parasites are found predominantly in a particular species of mammal or a group of related animals, and many of them were previously considered *C. parvum* (255). Even within *C. canis*, there are genetic differences among parasites in dogs, coyotes, and foxes. More host-adapted species and genotypes are likely to be present in mammals, since numerous intestinal *Cryptosporidium* genotypes in storm water cannot be attributed to specific animals (246).

Host adaptation probably also occurs in *Cryptosporidium* spp. of reptiles and birds, but to a much less extent. However, this may be due to the paucity of studies. As mentioned, minor genetic differences exist between *C. serpentis* from snakes and lizards. Unlike species or genotypes in mammals, each of which is usually seen in a very narrow range of animals, each *Cryptosporidium* species or genotype identified thus far in reptiles apparently can infect a broad range of reptilian hosts. Thus, both *C. serpentis* and *C. saurophilum* are seen in both snakes and lizards, and the tortoise genotype infects both tortoises and turtles (255; Xiao, unpublished). This also seems to be true for *Cryptosporidium* in birds. *C. baileyi*, *C. meleagridis*, and *C. galli* are all found in many different birds, although ducks and geese seem to have host-adapted genotypes (135, 143, 255).

Implications for Taxonomy

Understanding host adaptation and host-parasite coevolution is important to the revision of *Cryptosporidium* taxonomy. Recently, the validity of several host-adapted *Cryptosporidium* spp., such as *C. wrairi*, *C. felis*, *C. canis*, *C. hominis*, and *C. andersoni*, has been supported by cross-transmission studies. The former four were originally classified as *C. parvum.* For example, *C. wrairi* has been accepted as a valid species because of its difference in host specificity from *C. parvum* senso stricto. The same is also true for *C. andersoni*, originally considered to be *C. muris* but now established as a separate species because of differences in infectivity in cross-transmission studies (111). Prior experience with these newly established species suggest that many of the host-adapted genotypes would exhibit significant differences in infectivity in cross-transmission studies, in addition to the genetic differences greater than or comparable to those between established species. They will probably warrant species designation pending morphologic and biological characterizations. In addition, host adaptation indicates the existence of natural segregation and barriers for genetic recombination.

However, not all host-adapted genotypes should be considered separate species. The *Cryptosporidium* parasite in coyotes and one of the two genotypes in foxes are genetically very closely related to the *C. canis* in dogs and should be considered variants of *C. canis.* The same is probably true of the monkey genotype, which is almost identical to *C. hominis.* The challenge is in determining which are species and which are variants of the same species. For example, even though the marsupial genotype from Australia and the opossum genotype I in North America have been separated from each other for more than 50 million years and have a genetic distance greater than that between members mentioned above, these two parasites are clearly related. There are even quite big genetic differences among marsupial genotype isolates in Australia. In such cases, biological studies are clearly needed to resolve the taxonomic problem.

CRITERIA FOR NAMING *CRYPTOSPORIDIUM* SPECIES

Because of the difficulties associated with the taxonomy of *Cryptosporidium* spp., general guidelines should be developed as an aid in establishing new species of *Cryptosporidium.* The fourth edition of ICZN, which governs the taxonomy of protozoan parasites and other animals, took effect on 1 January 2000. Within the introduction, Ride et al. (192) list a series of underlying principles that are pertinent to this discussion. The first principle provides an overall foundation for discussion, and states "The Code refrains from infringing upon taxonomic judgment, which must not be made subject to regulation or restraint." Loosely translated, this means that the criteria used in establishing any new species depend on what experts in each individual field accept as valid criteria. Therefore, there are no set international guidelines for establishing a new species of *Cryptosporidium* per se, providing that the 90 articles of ICZN are not violated. Therefore, the real question is whether we should, as *Cryptosporidium* researchers, establish or recommend general guidelines for describing new species of *Cryptosporidium.* Based on the plethora of synonyms, nomen nuda, and genetically cryptic species that seem to be hidden within the genus, recommendations for naming new species of *Cryptosporidium* seem to be in order.

At the 6th Meeting on Molecular Epidemiology and Evolutionary Genetics in Infectious Disease at the Pasteur Institute in Paris, France, a session was held entitled "The taxonomy of the genus *Cryptosporidium.*" The objective of this session was to review the current criteria used to name species of *Cryptosporidium*, to evaluate the merits of these criteria, and to propose the most suitable criteria for future use. The panelists discussed areas of concern and provided recommendations for improvement. It was suggested that when naming new species of *Cryptosporidium*, four basic requirements should be fulfilled: (i) morphometric studies of oocysts; (ii) genetic characterizations; (iii) demonstration of natural and, whenever feasible, at least some experimental host specificity; and (iv) compliance with ICZN.

Oocyst Morphology

Even though oocysts of many *Cryptosporidium* spp. are morphologically similar, morphometric measurement of oocysts

TABLE 3. *Cryptosporidium* spp. of humans, domestic animals, and some wildlife

Host	Major parasite(s)	Minor parasite(s)
Human	*C. hominis*, *C. parvum*	*C. meleagridis*, *C. felis*, *C. canis*, *C. muris*, cervine genotype, pig genotype I
Cattle	*C. parvum*, *C. andersoni*	Bovine genotype B, deer-like genotype, *C. felis*
Sheep	*C. parvum*	Bovine genotype B, cervine genotype (sheep genotype?), *C. andersoni*, *C. hominis*
Goat	*C. parvum*	*C. muris* (moutain goat)
Camel	*C. parvum*?, *C. andersoni*	*C. muris*
Pig	Pig genotype I	Pig genotype II
Horse	Horse genotype?	Apparently *C. parvum*
Dog	*C. canis*	
Cat	*C. felis*	
Mouse	*C. muris*, mouse genotype	
Squirrel	Squirrel genotype, *C. muris*	
Deer	Deer genotype, *C. parvum*	Cervine genotype
Muskrat	Muskrat genotype I	Muskrat genotype II
Opossum	Opossum genotypes I and II	
Fox	*C. canis* fox genotype	Fox genotype II, *C. canis* dog genotype
Chicken	*C. baileyi*	*C. meleagridis*, *C. galli*
Turkey	*C. baileyi*, *C. meleagridis*	
Goose and duck	Goose genotypes I and II	*C. baileyi*, duck genotype
Snake	*C. serpentis*	*C. saurophilum*, snake genotype (W11)
Lizard	*C. serpentis*, *C. saurophilum*	Lizard genotype
Turtle	Tortoise genotype	

can play a vital role in the differentiation of some *Cryptosporidium* spp. For example, the established species in birds and reptiles can easily be differentiated on the basis of the size and shape of oocysts. Even among the intestinal species in mammals, there are significant differences in morphometrics (Table 1). Therefore, each species description should be accompanied by a series of morphologic measurements of a population of oocysts, usually 20 to 100 oocysts, complete with the means, ranges, and sometimes standard deviations or confidence limits of the measurements (length, width, and shape index or length/width ratio). Whenever possible, oocysts should be excysted and the size of sporozoites should be measured.

Genetic Characterizations

We can no longer describe new *Cryptosporidium* species solely based on morphologic descriptions or developmental studies. Most animals can be naturally infected with multiple *Cryptosporidium* spp. (Table 3), which frequently cannot be differentiated from each other on the basis of morphology and development (Table 1; Fig. 1). To avoid confusion in the identity of the parasites involved, defining distinct genetic differences between species will be key when naming new species of *Cryptosporidium.*

Unfortunately, the fourth edition of the ICZN does not address the use of genetic sequences when defining new species. The only related point that is discussed is the concept of genotype, the use as a type of which is dismissed in Article 67, Recommendation 67A (192). This recommendation understandably states ". . .To avoid ambiguity the term "genotype," which has widespread use in a different set of genetics, should not be used instead of "type species." " Thus, where genetic sequences in GenBank or other electronic databases, cloned genes, or cDNA and gDNA libraries deposited in accredited museums such as the American Type Culture Collection fit into the type specimen concept has yet to be determined. It is also unclear whether these sequences should be regarded as representing syntypes or whether they better fit under the hapantotype concept (Article 72.5.4: "in extant species of protistans, one or more preparations of directly related individuals representing differing stages in the life cycle"). Because it is up to each perspective field to establish its own criteria for species designation, it is not a violation of ICZN to use genetic data as an aid in *Cryptosporidium* taxonomy. However, it should be noted that genetic data cannot be used as the sole criterion in naming new *Cryptosporidium* spp. Not only are there no provisions in the ICZN for this, but also the possibility always exists that key genetic sequences used in a species description may become obsolete as new species and isolates are discovered.

If nucleotide sequences are to play a pivotal role in differentiating species of *Cryptosporidium*, two key points should be examined. First, which of the genes and/or noncoding regions should be used in the taxonomic descriptions? Second, how much variability constitutes a separate species? The first query is most easily addressed, although it is not without controversy. In most instances, common nucleotide sequences such as SSU rRNA, actin, and HSP70 have been employed, with SSU rRNA being the most commonly used. These sequences are useful not only because they are universal in distribution but also because generic primers can be employed. Recent examples where common sequences were used include the differentiation of *C. andersoni* from *C. muris*, the differentiation of *C. canis* from *C. parvum*, and, most recently, the establishment of *C. hominis* from humans as a species distinct from *C. parvum* (63, 111, 146). However, other sequences have also been used to great effect in differentiating some species of *Cryptosporidium*, despite the potential for greater difficulties in primer design. These include genes encoding the oocyst wall protein (COWP [250]), thrombospondin-related adhesive protein 1 (TRAP-C1 [200]), and tubulin (32, 194, 210, 243), to name but a few, as well as noncoding sequences such as the internal transcribed spacer 1 (147) and microsatellites (1, 31, 64). Differences in sequences encoding microneme and rhoptry pro-

TABLE 4. Some primers used in the characterization of the SSU rRNA, HSP70, and actin genes of various *Cryptosporidium* spp.

Gene	Primers		Amplicon size (bp)	Usage	Reference
	Name	Sequence (5′ to 3′)			
SSU rRNA	SSU-F1	AACCTGGTTGATCCTGCCAGTAGTC	~1,750	Amplify the full rRNA gene of most eukaryotic organisms	256
	SSU-R1	TGATCCTTCTGCAGGTTCACCTACG			
SSU rRNA	SSU-F2	TTCTAGAGCTAATACATGCG	~1,325	Both sets of primers are *Cryptosporidium* specific and can be used in nested PCR	246, 256
	SSU-R2	CCCATTTCCTTCGAAACAGGA			
	SSU-F3	GGAAGGGTTGTATTTATTAGATAAAG	~820		
	SSU-R3	AAGGAGTAAGGAACAACCTCCA			
HSP70	HSP-F1	ATGTCTGAAGGTCCAGCTATTGGTATTGA	~2,010	Both sets of primers amplify most apicomplexan parasites and are used in nested PCR	213
	HSP-R1	TTAGTCGACCTCTTCAACAGTTGG			
	HSP-F2	TA/CTTCATG/CTGTTGGTGTATGGAGAAA	~1,950		
	HSP-R2	CAACAGTTGGACCATTAGATCC			
Actin	Act-F1	ATGA/GGA/TGAAGAAGA/TAA/GC/TA/TCAAGC	~1,095	Both sets of primers amplify most apicomplexan parasites and are used in nested PCR	211
	Act-R1	AGAAG/ACAC/TTTTCTGTGT/GACAAT			
	Act-F2	CAAGCA/TTTG/AGTTGTTGAT/CAA	~1,066		
	Act-R2	TTTCTGTGT/GACAATA/TG/CA/TTGG			

teins, such as gp900 (also known as polythreonine protein or poly-T [19, 35]), were identified between *C. parvum* and *C. hominis* (35, 212). They may eventually be shown to be pronounced in some isolates and should reflect adaptation of the parasite to specific hosts. Although designing primers against these more variable sequences may prove too difficult (213), they may also, in time, prove to be highly useful in species recognition and pathogenesis studies.

In addressing the second point, which attempts to make predictions about the degree of variability to be used to define species, there can be no easy answer. Even with protists such as *Cryptosporidium*, intraspecific allelic variation occurs and these differences will vary depending on the nucleotide sequence being studied. Recently, over 75,000 common chimpanzee sequences were aligned and mapped to the human genome, resulting in the discovery of 98.77% genetic similarity between the two vertebrates (245). Thus, sometimes a high degree of genetic similarity can exist even when we are dealing with separate genera. To be cautious, one would suspect that a new species is involved if the genetic differences between two isolates are greater than or comparable to those between established *Cryptosporidium* spp. However, when genetic differences are small, then careful biological studies (developmental biology, host specificity in cross-transmission studies, predilection site, prepatent and patent periods, intensity of oocyst shedding, virulence, etc.) with multiple isolates have to be used to support genetic findings. It cannot be overstated that genetic studies in themselves cannot be used as the sole means of differentiating species of *Cryptosporidium.* Not only are there no provisions within the ICZN for this, but also newer species may eventually be found to possess identical genetic sequences in some regions, effectively invalidating a genetically defined species.

A mere description of the percent differences or numbers of nucleotide changes in genetic sequences is also not enough when dealing with species description. For comparisons with other species and genotypes, DNA sequence data should be presented in the original paper and/or deposited in public databases (38). Precise areas of nucleotide differences that help define a new species should be clearly defined, and the genetic relationship between the new and existing parasites should be examined by phylogenetic analysis. Therefore, it is important that sizeable DNA fragments of the selected genes should be amplified and informative regions should be targeted. This is especially important for genes with polymorphic regions interspersed between conserved regions, such as the SSU rRNA gene. To avoid PCR contamination and misinterpretation of data, more than one genetic locus should be characterized. Different genes or markers evolve at different rates, and this can produce different results. Genetic diversity exists within an individual *Cryptosporidium* species or genotype, and different copies of the SSU rRNA gene have minor sequence differences in some intestinal *Cryptosporidium* spp. (*C. parvum*, *C. hominis*, *C. felis*, marsupial genotype, opossum genotype I, and others). Multilocus characterizations can help to determine whether these observed sequence differences represent genetic differences at the species level. Table 4 lists some of the primers commonly used for the characterization of the SSU rRNA, actin, and HSP70 genes of *Cryptosporidium* parasites. Occasionally, the genetic differences between two parasites are significant at all genetic loci examined yet the two parasites are clearly related, such as the marsupial genotype and opossum genotype I.

Natural Host Specificity

Infectivity in cross-transmission studies has often been used as an important criterion in *Cryptosporidium* taxonomy. Nev-

ertheless, because significant biological variations exist among isolates of the same *Cryptosporidium* species, various parasite, host, and laboratory factors all help control the susceptibility of a host animal to infection. Only a few species of laboratory animals are in common use, and the usefulness of host specificity as demonstrated in cross-transmission studies in naming new *Cryptosporidium* species is sometimes limited. There are already conflicting and confusing data regarding host specificity of *C. andersoni*, *C. hominis*, *C. wrairi*, and *C. felis.* Nevertheless, the extensive data accumulated thus far indicate that in addition to generalist species like *C. muris*, host-adapted *Cryptosporidium* spp. exist, which should be useful for studying natural host specificity and barriers to genetic recombination. Therefore, when naming new *Cryptosporidium* species, it is suggested that researchers should determine the spectrum of host animals within limits of feasibility and compare those isolates with established species. This may seem like a daunting task, but with the ever-increasing amount of molecular characterizations of *Cryptosporidium* isolates from different animals, it may be as simple as tabulation of the spectrum of host animals infected with an isolate based on previously published literature. Researchers should refrain from naming new *Cryptosporidium* species based solely on the finding and characterization of one or two isolates.

Compliance with ICZN

In recommending general criteria for naming new species of *Cryptosporidium*, at least two points within the ICZN should be emphasized. First, Articles 8 and 9 of the ICZN spell out clear criteria for publication (192). Most conspicuous is Article 9, which lists formats not constituting valid, published work. These include microfilm, unpublished copies of work even if available in a library or archive, material in the form of electronic signals on the World Wide Web, tape recordings, and abstracts or papers issued primarily to participants at meetings or symposia. Thus, the clear mandate in the ICZN is that the International Committee still prefers taxonomic descriptions to be printed on paper, although publication in the form of CD-ROM appears to be acceptable if the work has ". . .been deposited in at least 5 major publicly accessible libraries which must be identified by name within the work itself."

The second point pertains to Article 72.3 which states, "Name-bearing types must be fixed originally for nominal species-group taxa established after 1999." Therefore, in order for a species of *Cryptosporidium* to be valid under the ICZN, the authors must establish a name-bearing type in the form of a holotype or designated syntypes. Because of the difficulty of fixing single (holotype) specimens as name-bearing types for most members of the Apicomplexa, specimens in a type series (syntypes) are almost always used (Article 72.1.1). For coccidia and *Cryptosporidium* spp., the morphological description itself and the museum photographs of the unpreservable oocysts (sometimes termed phototypes [17]) should be provided. Whenever possible, stained slides and histological sections should be deposited. The museum in North America where the majority of type specimens currently reside is the U.S. National Parasite Collection in Beltsville, Md. It should be noted that photographs and illustrations are not types themselves but, rather, a representative of the name-bearing type (Article 72.5.6).

Currently *Cryptosporidium* species and genotypes have been named or identified primarily in association with the host from which they were isolated. This can be problematic, since most *Cryptosporidium* spp. infect more than one species of animals. Therefore, the initial host identified may not be the host the parasite most commonly infects. Also, usually more than one *Cryptosporidium* species may be commonly found in one host species. For example, if *C. meleagridis* were first found in a mammal instead of turkeys, it would have been named differently.

PUBLIC HEALTH IMPORTANCE OF *CRYPTOSPORIDIUM* TAXONOMY

Cryptosporidiosis is a major public health problem in both developing and developed countries. In developing countries, the disease probably exerts most of its impact on pediatric health. In addition to the occurrence of diarrhea, cryptosporidiosis has been attributed to malnutrition and stunted growth (41, 42, 126). In developed countries, waterborne outbreaks of cryptosporidiosis have a significant economic impact. A recent study estimates that the total illness-associated cost of the 1993 Milwaukee waterborne outbreak of cryptosporidiosis is $96.2 million: $31.7 million in medical costs and $64.6 million in productivity losses. The average total costs for a person with mild, moderate, and severe illness during the outbreak are $116, $475, and $7,808, respectively (46). These cost estimates do not include litigations and infrastructure improvements in water treatment facilities after the outbreak. Pretty et al. (189) have estimated that the United Kingdom will expend an average of £23 million/year to meet legal requirements for removal of *Cryptosporidium* from drinking water supplies. Therefore, it is important for researchers to determine the extent of contamination of surface waters with species of *Cryptosporidium* infective for humans. Understanding the taxonomy of *Cryptosporidium* spp. can be also useful in establishment of the identity of the parasites infecting humans, assessment of the public health significance of *Cryptosporidium* from animals and the environment, and tracking of infection and contamination sources. All these will promote understanding of the transmission and epidemiology of human cryptosporidiosis, development of preventive measures to minimize exposures to infections, accurate risk assessment, and scientific management of the watershed.

Identity of *Cryptosporidium* Species in Humans

For quite some time, it was thought that *C. parvum* was the species responsible for human cryptosporidiosis. This is largely due to a lack of clear understanding of the species structure of the genus *Cryptosporidium* and the reliance on morphologic features for differentiation. With the use of genetic analysis in helping define species, we now have a better knowledge of the taxonomy of *Cryptosporidium* spp. and the molecular tools to differentiate species with similar oocyst morphology. Thus, numerous studies have shown two *Cryptosporidium* spp., *C. parvum* and *C. hominis*, to be responsible for most human *Cryptosporidium* infections (5–7, 15, 16, 25, 26, 31, 32, 35, 37, 68, 72,

TABLE 5. Prevalence of five common *Cryptosporidium* spp. in humans[a]

Location	Type of patients	Total no. of patients	No. of patients infected with:					Reference(s)
			C. hominis	*C. parvum*	*C. meleagridis*	*C. felis*	*C. canis*	
Portugal	HIV$^+$	29	7	16	3	3	0	8
Switzerland	HIV$^+$	13	2	7	1	3	0	127
France	HIV$^+$	46	14	22	3	6	0	82
Thailand	HIV$^+$	29	24	0	3	1	0	216
Thailand	HIV$^+$	34	17	5	7	3	2	71
Atlanta	HIV$^+$	10	5	1	0	3	1	187
New Orleans	HIV$^+$	29	18	8	0	3	0	249
Peru	HIV$^+$	118	76	20	10	4	9	249
Peru	Children	83	65	8	7	1	2	248
Kenya	All	33	23	8	1	0	0	70
Japan	All	22	16	3	3	0	0	258
United Kingdom	All	1,680–2,057	815	1,247	19	4	1	177, 179, 180

[a] Only data from studies using PCR that amplifies all five *Cryptosporidium* spp. are quoted.

76, 82, 83, 120, 121, 127, 130–134, 136, 161, 166, 168, 179, 183, 185, 194, 199–201, 206, 209–213, 235, 241–244, 248, 250, 254, 256, 257). Subsequently, after the identification of the genetic uniqueness of *C. meleagridis*, *C. felis*, and *C. canis* (137, 256, 257), these three parasites, which are traditionally associated with animals, were found in AIDS patients in the United States, Kenya, and Switzerland (127, 128, 187). More recent studies have confirmed the presence of these parasites in immunocompromised individuals in other parts of the world (5, 6, 71, 82, 216) (Table 5). Other *Cryptosporidium* spp. found in humans include *C. muris*, the cervine genotype, and pig genotype I (69, 82, 165, 169, 216, 247). The last three *Cryptosporidium* spp., however, have a very low prevalence in humans and are unlikely to emerge as major human pathogens. Immunosuppression is apparently not a prerequisite for infections with any of these species since they have been found in immunocompetent persons in Peru, the United Kingdom, and Japan (177, 178, 180, 248, 258). In Peru, where a significant proportion of infections in humans are due to zoonotic *Cryptosporidium* spp., there is no significant difference between children and HIV$^+$ adults in the distribution of all five common *Cryptosporidium* parasites (*C. hominis*, *C. parvum*, *C. meleagridis*, *C. felis*, and *C. canis*) (249).

Although seasonal variations occur, *C. hominis* tends to be responsible for most outbreaks of human cryptosporidiosis in many regions of the world (70, 71, 100, 140, 165, 166, 185, 209, 216, 248, 258). However, in Europe, *C. parvum* is often the dominant parasite in humans (5 to 7, 82, 120, 127) (Table 5). It seems likely, but remains unproven, that the expansion of *C. parvum* into humans in some regions of the world may be due, in part, to the intensive husbandry practiced for ruminants and the associated high concentrations of young animals at these feeding operations. The recent finding in humans of *C. meleagridis*, which is responsible for more human infections than was previously thought (71, 248), indicates that host expansion by *C. meleagridis* is also of public health significance. We should be aware that some of the other *Cryptosporidium* spp. not yet found in humans may also have a broad host range and may emerge as new pathogens in humans when socioeconomic and environmental changes favor transmission.

Significance of *Cryptosporidium* Species in Animals and the Environment

The host-adapted nature of most *Cryptosporidium* spp. indicates that the majority of species probably do not have high infectivity for humans. Thus, *Cryptosporidium* spp. commonly found in reptiles and most wild mammals have never been detected in humans and probably have no significant public health importance. On the other hand, host adaptation is not strict host specificity. A species or genotype may preferentially infect a species or group of animals, but this does not mean that this parasite cannot infect other animals. As mentioned above, *C. parvum*, *C. meleagridis*, *C. felis*, *C. canis*, *C. muris*, the cervine genotype, and pig genotype I have all been found in humans. Others, such as *C. andersoni* in cattle, pig genotype II, the mouse genotype in rodents, and *C. baileyi*, *C. galli*, and goose genotypes I and II, have not been found in humans. Thus, parasites from different animals and different species from the same animal have vastly different zoonotic potentials. More often, a *Cryptosporidium* species or genotype is found in a few different animals, and one species of animal is usually susceptible to multiple *Cryptosporidium* spp. (Table 3). Thus, all species of *Cryptosporidium* infect a limited range of animals (Table 1), and when the host range or infectivity includes humans, the parasite acquires public health significance.

The development of genetic tools as part of the taxonomic characterization of *Cryptosporidium* spp. makes it possible to directly assess the human infection potential of *Cryptosporidium* oocysts found in the environment. Currently, the identification of *Cryptosporidium* oocysts in environmental samples is based largely on the immunofluorescence assay (IFA) after concentration processes (ICR method, method1622/1623, flow cytometric method, solid-phase cytometric method [106]). Because IFA detects oocysts from all species of *Cryptosporidium*, the species distribution of *Cryptosporidium* in environmental samples cannot be assessed. Although many surface water samples contain *Cryptosporidium* oocysts, it is unlikely that all of these oocysts are from human-pathogenic species or genotypes, because only five *Cryptosporidium* spp. (*C. parvum*, *C. hominis*, *C. meleagridis*, *C. canis*, and *C. felis*) are responsible for most human *Cryptosporidium* infections. Information about the identity of *Cryptosporidium* oocysts is necessary for accu-

TABLE 6. Detection of *Cryptosporidium* in natural water by PCR-based molecular techniques

Method[a]	Gene target	Vol of water (liters)	No. of positive samples/total no. of samples	Species or genotype[b]	Reference
CC-PCR	HSP70	10	Source water: 6/122 Backwash water: 9/121	7 sequence types, probably from *C. parvum*, *C. hominis*, and mouse genotype	51
IMS-nested PCR-RFLP	SSU rRNA	189–224 (IMS done on Percoll-sucrose concentrates)	Storm water: 27/29	12 species/genotypes, all from wildlife	246
IMS-nested PCR	TRAP-C2	500–1,000	Surface and finished water: 11/214	*C. parvum*	113
IMS-PCR	SSU rRNA and TRAP-C2	500	River water and sewage effluent: 2/10	*C. parvum*	114
IMS-nested PCR-RFLP	SSU rRNA	Surface water: 10–63.1 Wastewater: 0.01–0.50	Surface water: 25/55 Raw wastewater: 12/49	Surface water: *C. andersoni* (5), *C. hominis* (10), *C. parvum* (19), *C. baileyi* (1) Wastewater: *C. andersoni* (8), *C. hominis* (1), *C. parvum* (1), *C. canis* (1), *C. muris* (1), *C. felis* (1), cervine genotype (1)	253
RT-PCR	HSP70	2.5–10	River water: 2/6	*C. parvum* (1); *C. parvum* and *C. meleagridis* (1)	95
IMS-nested PCR	SSU rRNA	40–80	Surface water: 7/78	*C. parvum*? (3), *C. andersoni* (4), *C. baileyi*? (1)	92
IMS-nested PCR	SSU rRNA	2 or 20	Surface water: 24/60 Wastewater: 6/8	*C. parvum* (6) and *C. hominis* (4), *C. muris* (6), *C. andersoni* (3), *C. baileyi* (1), 3 new genotypes (3), dinoflagellates (7)	238

[a] Methods detect only *C. parvum*, *C. hominis*, *C. meleagridis*, and closely related parasites. CC-PCR, cell culture PCR; IMS, immunomagnetic separation.
[b] Number of samples where the species was detected is given in parentheses.

rate risk assessment of contamination in water. Thus, identification of oocysts to species and genotypes is of public health importance.

Several species-differentiating and genotyping tools have been used to determine whether *Cryptosporidium* oocysts found in water are from human-infective species (Table 6). An SSU rRNA-based nested PCR-RFLP method has been successfully used for the detection and differentiation of *Cryptosporidium* oocysts present in storm water, raw surface water, and wastewater (246, 253). In one study, 29 storm water samples were collected from a stream that contributes to the New York City water supply system and were analyzed for *Cryptosporidium* oocysts. Twelve wildlife genotypes of *Cryptosporidium* were detected in 27 of 29 positive samples. None of the genotypes have ever been found in humans and therefore probably do not infect humans. Of the 27 PCR-positive samples, 12 contained multiple genotypes (246).

In another study, the same technique was used in the analysis of raw surface water samples collected from different locations (Maryland, Wisconsin, Illinois, Texas, Missouri, Kansas, Michigan, Virginia, and Iowa) in the United States and produced quite different results. A total of 55 samples were analyzed, 25 of which produced positive PCR amplification. Only four *Cryptosporidium* spp. (*C. parvum*, *C. hominis*, *C. andersoni*, and *C. baileyi*) were found, two of which are known human pathogens. Similar results were also obtained from 49 samples of raw urban wastewater collected from a treatment plant in Milwaukee, Wis., 12 of which were positive. Seven *Cryptosporidium* spp. (*C. parvum*, *C. hominis*, *C. andersoni*, *C. muris*, *C. canis*, *C. felis*, and the cervine genotype) were found, with *C. andersoni* as the most common species; this species is not a human pathogen. As expected, the diversity of *Cryptosporidium* spp. found in source waters and wastewaters was much lower than in storm waters (253).

Promising results in genotyping *Cryptosporidium* spp. in water samples have also been generated in recent studies using other techniques (Table 6). HSP70 sequence analysis of PCR-amplified cell culture products revealed the presence of six sequence types of *C. parvum* in raw surface water samples and filter backwash water samples (51). Comparison of these sequences with the HSP70 sequences collected from various *Cryptosporidium* spp. indicates that these sequences were from *C. parvum*, *C. hominis*, and the mouse genotype (213). Analysis of six river water samples by an HSP70-based reverse transcription-PCR technique also showed the presence of *C. parvum* and *C. meleagridis* in two samples (95), even though the primers used in the study were previously shown to have poor specificity (96).

Two SSU rRNA-based PCR sequencing tools have also been successfully used in the differentiation of *Cryptosporidium* oocysts in surface water and wastewater samples (92, 238). Sequences of *C. andersoni* and presumably *C. parvum* and *C. baileyi* were obtained from seven samples of surface water from a watershed in Massachusetts (82). Analysis of 17 positive surface water samples and 6 wastewater samples from Germany and Switzerland showed the presence of eight *Cryptosporidium* genotypes, with *C. parvum*, *C. hominis*, *C. muris*, and *C. andersoni* being the most prevalent parasites, and showed that 4 samples contained three unidentified wildlife genotypes and *C. baileyi* (238). Using sequencing analysis of TRAP-C2, *C. parvum* was also found in 11 of 214 surface and finished water samples in Northern Ireland in one study and in 2 of 10 river

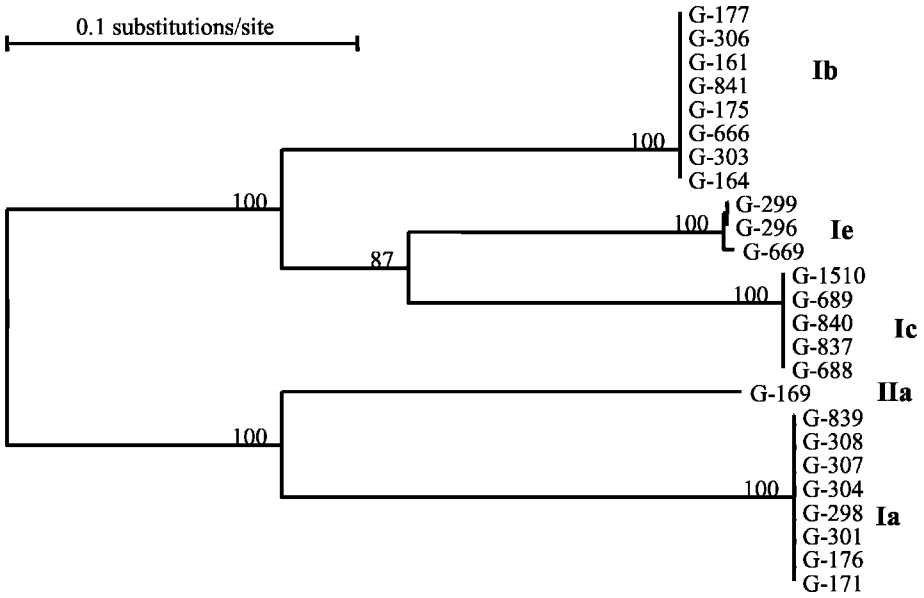

FIG. 3. Genetic diversity in *C. parvum* and *C. hominis* from AIDS patients in New Orleans, based on neighbor-joining analysis of the partial GP60 gene. Values on branches are percent bootstrapping using 1,000 replicates. Five allele families of parasite are seen: Ia, Ib, and Ie are *C. hominis* allele families, and Ic and IIa are *C. parvum* allele families. Modified from reference 249.

water and sewage effluent samples in another study (113, 114). Results of these recent studies support the conclusion that many *Cryptosporidium* spp. found in water do not have high potential for infecting humans.

Infection and Contamination Sources

The host-adapted nature of most *Cryptosporidium* spp. makes it possible to track the source of *Cryptosporidium* infection in humans and oocyst contamination in the environment. The high prevalence of *C. hominis* in humans indicates that humans are a major source of infection for human cryptosporidiosis. The finding of *C. parvum*, *C. meleagridis*, *C. felis*, *C. canis*, *C. muris*, pig genotype I, and cervine genotype in humans suggests that farm animals, domestic pets, and some wildlife can be potential sources. Indeed, direct transmission of *C. parvum* from animals to humans is well documented (48, 124, 125, 185, 204). Many food-borne and waterborne outbreaks of cryptosporidiosis have been caused by *C. parvum* in North America and Europe (120, 166, 185, 209), even though it is frequently unclear whether humans or animals are the source of contaminations.

Caution should be used in interpreting the significance of finding parasites traditionally associated with animals in humans. It is always tempting to attribute the source of *C. parvum* and other zoonotic *Cryptosporidium* spp. found in humans and the environment solely to animals (166, 204). Contradictory to what may be intuitive, many human *C. parvum* infections in localized areas may indeed have originated from humans themselves. This is supported by sequence analysis of the GP60 gene, a sporozoite surface glycoprotein of *C. parvum* and *C. hominis*. As shown in Fig. 3, five of the six *C. parvum* isolates from AIDS patients in New Orleans possessed the allele family Ic (249). Likewise, all *C. parvum* isolates (>50 isolates) from children and AIDS patients in Peru also exhibited this allele family (Xiao, unpublished). This allelic type was apparently also the dominant type in South Africa (100), and 5 of 16 *C. parvum* isolates from Portuguese AIDS patients had this allele (8). In contrast, allele family Ic has never been found among over 500 bovine *C. parvum* isolates analyzed thus far in the United States, Portugal, and the United Kingdom (8, 74, 184; L. Xiao and J. E. Moore, unpublished data). It was previously suggested that GP60 allele family Ic resulted as a recombination between the *C. parvum* allele families IIa and *C. hominis* allele Ib (100). However, among the GP60 allele families of *C. parvum* and *C. hominis*, *C. parvum* allele family Ic appears phylogenetically related to *C. hominis* allele families Ib and Ie whereas *C. parvum* allele family IIa is related to *C. hominis* allele families Ia and Id (Fig. 3). The pattern of sequence diversity in allele family is also different from that in all other *C. parvum* and *C. hominis* allele families. It remains to be determined whether the genetic and biological uniqueness of allele Ic is truly due to sexual recombination. In any case, the previously identified two transmission cycles (the anthroponotic transmission cycle with *C. hominis* and the zoonotic transmission cycle with *C. parvum*) of cryptosporidiosis in humans is apparently a simplistic view of the complexity of *Cryptosporidium* infection.

The same is probably also true for *Cryptosporidium* contamination in water. *C. parvum* is one of several common *Cryptosporidium* spp. found in water, but it is difficult to determine the source of *C. parvum* oocysts in water. Nevertheless, the high prevalence of *C. andersoni* oocysts in surface water and even wastewater indicates that ruminants are probably a major source of *Cryptosporidium* contamination. The finding of *C. baileyi*, *C. muris*, and other unknown *Cryptosporidium* genotypes in water in several studies indicates that wildlife also contribute to *Cryptosporidium* oocyst contamination in water (92, 238, 246).

C. hominis is also frequently seen in surface water in some areas and is generally considered a relatively strict human pathogen. However, most *Cryptosporidium* spp. identified in mammals have preferred host taxa but also infect multiple hosts to lesser degrees. Indeed, *C. hominis* has been identified recently in a few animals, such as a dugong and a sheep (73, 142). Lambs, calves, and gnotobiotic pigs have also been successfully infected with some isolates of *C. hominis* (3, 55, 73, 186). Recently, some wetland mammals such as beavers and muskrats have been identified as reservoir hosts for *Giardia intestinalis* and *Enterocytozoon bieneusi* infections in humans by genetic analysis (207, 208). Similarly, an undiscovered zoonotic reservoir for *C. hominis* may also exist.

Implications for the Water Industry

The water industry should take comfort in knowing that even though oocysts of all *Cryptosporidium* spp. can potentially appear in water, only a few of them are known human pathogens. Even among the latter, not all have the same infectivity for humans. Very limited studies have been conducted to determine the identity of *Cryptosporidium* oocysts in water, but the results generated thus far indicate that a large proportion of *Cryptosporidium* oocysts in water are not from species harmful to humans. Current detection methods for *Cryptosporidium* oocysts in water do not include a species differentiation process. Therefore, the human health impact could be overestimated if the risk assessment models do not take this into consideration. More studies involving systematic sampling of different types of water are clearly needed to develop a better picture of the extent of contamination of water with human-infective *Cryptosporidium* spp. in different environmental settings.

Currently, surface water in the United States is frequently used as drinking water after conventional treatment, which includes coagulation, flocculation, sedimentation, filtration, and chlorination. When working properly, this process is very effective in removing *Cryptosporidium* oocysts (88). Therefore, determining the species nature and human-infective potentials of *Cryptosporidium* oocysts in the source water has not been a high-level concern, especially when the treatment plant also incorporates ozone or uv light treatment near the end of the treatment process. In some areas, however, surface water is treated only by chlorination or is processed by partial treatment without filtration. Under such circumstances, it is critical to minimize the presence of human-pathogenic *Cryptosporidium* spp. in source water. Thus, the use of surface water potentially contaminated by human and agricultural activities as source water is problematic because humans and farm animals are major sources of contamination by oocysts of human-pathogenic *Cryptosporidium* spp. In contrast, for water utilities that use pristine water as the source water, partial treatment of surface water may indeed be enough, since *Cryptosporidium* oocysts in these watersheds are from species originating from wildlife, which are mostly not human pathogens. Thus, periodic determination of the species of *Cryptosporidium* oocysts in any watershed or source water may be very helpful in the development of strategies for the scientific management and protection of source water.

CONCLUDING REMARKS

The taxonomy of *Cryptosporidium*, like the taxonomy of most organisms, remains fluid. Even though new criteria may be introduced in future, the taxonomic framework discussed above should be helpful in guiding us to minimize the creation of invalid names for species. As with the revision of the taxonomy of any pathogen, the above suggestions will not be without controversy. However, the goal of this paper is to put forth a scheme that incorporates taxonomic characteristics based on classical methods with newly emerging techniques. When combined, these data should prove useful in understanding the biology, epidemiology, and public health importance of various *Cryptosporidium* spp. With the separation of *C. hominis* and, shortly, the mouse genotype from *C. parvum*, it is expected that some of the previous confusion will be eliminated.

In the meantime, we suggest that researchers follow the proposed guidelines in naming *Cryptosporidium* species. Thus, a description of new species should provide easily identifiable characteristics that enable other researchers to clearly differentiate this parasite from known *Cryptosporidium* species, and from other yet unnamed *Cryptosporidium* spp. that may be found in the same or related hosts. This, at minimum, should include detailed morphometric descriptions of the oocysts, comparative data about the natural host specificity or spectrum of known hosts, and results of genetic characterizations and should comply with all 90 articles of the ICZN. Past experience in the area indicates that excessive reliance on any single criterion or results of cross-transmission is risky and frequently results in the later invalidation of the described species.

Researchers should carefully use *Cryptosporidium* nomenclature to avoid unnecessary confusion. The name *C. parvum* should be used only for parasites previously known as the bovine genotype or genotype 2. Even though we have known for years that there is extensive genetic and biologic heterogeneity in mammalian *Cryptosporidium* isolates and that multiple *Cryptosporidium* spp. infect humans, too often researchers habitually refer to the parasites in humans or mammals as *C. parvum* without providing any evidence to support this identification. Therefore, unless the nature of these species is resolved by biological or molecular characterizations, it is scientifically incorrect to assign species names. In most such cases, the use of the term *Cryptosporidium* sp. or merely *Cryptosporidium* should be enough.

With the establishment of a framework for naming *Cryptosporidium* species and the availability of new genetic tools, there should be reduced confusion associated with the taxonomy of the genus. We also hope that the more detailed suggestions for naming new *Cryptosporidium* spp. will reflect the evolutionary relationships within the genus as well as providing a rational basis for delineating species. This, in turn, will promote the assessment of the public health importance of various species and isolates of *Cryptosporidium* and allow researchers to better understand the transmission dynamics, to identify risk factors and reservoir hosts, and to establish preventive measures.

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